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                      USPATFULL/USPAT2
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                      INPADOC
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 NEWS 21   SEP 25     CAS REGISTRY(SM) no longer includes Concord 3D coordinates
 NEWS 22   SEP 25     CAS REGISTRY(SM) updated with amino acid codes for pyrrolysine

 NEWS EXPRESS  JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 21 Sep 2006 (20060921/PD)
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USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Jun 2006


=> e haigwood n l/in
E1           1      HAIGOH HISAMITSU/IN
E2           1      HAIGRON MICHEL/IN
E3           0 --> HAIGWOOD N L/IN
E4          14      HAIGWOOD NANCY L/IN
E5           1      HAIHONG ZHENG/IN
E6           1      HAIJE JOHAN AREND FREDERIK/IN
E7           1      HAIJEMA BERT JAN/IN
E8           3      HAIJI HIROHISA/IN
E9           8      HAIJIMA AKIMITSU/IN
E10          3      HAIJIMA HIDEKI/IN
E11          3      HAIJIMA KAZUMI/IN
E12          1      HAIJIMA MIKIO/IN

=> s e4
L1          14 "HAIGWOOD NANCY L"/IN

=> d l1,cbib,clm,1-14

L1   ANSWER 1 OF 14  USPATFULL on STN
2005:298571 Aids vaccines.
    Haigwood, Nancy L., Seattle, WA, UNITED STATES
    Blay, Wendy, Seattle, WA, UNITED STATES
    Stamatatos, Leonidas, Seattle, WA, UNITED STATES
    US 2005260235 A1 20051124
    APPLICATION: US 2005-96698  A1  20050331 (11)
    PRIORITY: US 2004-558181P  20040331 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1. A vaccination protocol comprising administering to a primate host a
       first immunogen comprising at least one Human Immunodeficiency Virus
       type 1 Envelope (env) sequence having a first set of consensus
       glycosylation sequences, followed by a second immunogen comprising at
       least one primate immunodeficiency virus env sequence having a second
       set of consensus glycosylation sequences, wherein the differences
       between the first set of consensus glycosylation sequences and the
       second set of consensus glycosylation sequences comprise at least one
       of:  (a) an addition of a consensus N-linked glycosylation sequence in
       V1 at a position corresponding to position 143 or 144 in the HIV-89.6
       env sequence;  (b) a shift of a consensus N-linked glycosylation
       sequence in V2 from a position corresponding to position 186 in the
       HIV-89.6 env sequence to a position corresponding to position 188 in the
       HIV-89.6 env sequence;  (c) an addition of a consensus N-linked
       glycosylation sequence addition in C2 at a position corresponding to
       position 276 in the HIV-89.6 env sequence;  (d) an addition of a
       consensus N-linked glycosylation sequence addition in V4 at a position
       corresponding to position 386 in the HIV-89.6 env sequence;  (e) an
       addition of a consensus N-linked glycosylation sequence addition in C2
       at a position corresponding to position 397 in the HIV-89.6 env
       sequence; and  (f) a shift of a consensus N-linked glycosylation
       sequence in V5 from a position corresponding to position 460 in the
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HIV-89.6 env sequence to a position corresponding to position 462 or 463 in the HIV-89.6 env sequence.

L1 ANSWER 2 OF 14 USPATFULL on STN
2005:130679 Aids vaccines.
Haigwood, Nancy L., Seattle, WA, UNITED STATES
US 2005112138 A1 20050526
APPLICATION: US 2003-719004 A1 20031121 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A vaccination protocol comprising immunizing a mammalian host with a first HIV envelope immunogen having a minimum number of N- and O-linked glycosylation sites, followed by one or more booster immunizations with at least one second HIV envelope immunogen having more glycosylation sites than the first immunogen.

L1 ANSWER 3 OF 14 USPATFULL on STN
1999:136680 Peptide plasminogen activators.
Haigwood, Nancy L., Oakland, CA, United States
Mullenbach, Guy, Oakland, CA, United States
Afting, Ernst-Guenter, Marburg, Germany, Federal Republic of
Paques, Eric Paul, Marburg, Germany, Federal Republic of
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)Hoechst Marion Roussel Deutschland GmbH, Germany, Federal Republic of (non-U.S. corporation)
US 5976530 19991102
APPLICATION: US 1997-799823 19970213 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A human tissue plasminogen activator capable of dissolving clots having lysine 277 substituted with another amino acid, and further comprising a deletion of from 3 to 25 amino acids from the C-terminus.

2. A pharmaceutical composition comprising a human tissue plasminogen activator of claim 1.

3. A nucleic acid molecule encoding a human tissue plasminogen activator of claim 1.

4. A vector comprising the nucleic acid sequence of claim 3.

5. A method of making the vector of claim 4, comprising: (a) isolating said nucleic acid sequence; and (b) inserting said nucleic acid sequence into an expression vector such that it would be expressed in an appropriate host cell.

6. A host cell comprising the nucleic acid sequence of claim 3.

7. A method of making a human tissue plasminogen activator according to claim 1, comprising: (a) culturing a host cell comprising a nucleic acid encoding said human tissue plasminogen activator; and (b) isolating the protein.

8. A human tissue plasminogen activator of claim 1, further comprising a substitution of at least one of the group consisting of asparagine 117, asparagine 184 and asparagine 448 with another amino acid.

9. A pharmaceutical composition comprising a human tissue plasminogen

activator of claim 8.

10. A nucleic acid molecule encoding a human tissue plasminogen activator of claim 8.

11. A vector comprising the nucleic acid sequence of claim 10.

12. A method of making the vector of claim 11, comprising: (a) isolating said nucleic acid sequence; and (b) inserting said nucleic acid sequence into an expression vector such that it would be expressed in an appropriate host cell.

13. A host cell comprising the nucleic acid sequence of claim 10.

14. A method of making a human tissue plasminogen activator according to claim 8, comprising: (a) culturing a host cell comprising a nucleic acid encoding said human tissue plasminogen activator; and (b) isolating the protein.

L1 ANSWER 4 OF 14 USPATFULL on STN
1998:122538 DNA sequences encoding HIV-1 envelope muteins containing hypervariable domain deletions.
Haigwood, Nancy L., Oakland, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 5817792 19981006
APPLICATION: US 1995-441356 19950515 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A DNA sequence encoding a human immunodeficiency virus type 1 (HIV-1) envelope mutein having the structure C_1 -V_1 -V_2 -C_2 -V_3 -C_3 -V_4 -C_4 -V_5 -C_5 wherein said mutein retains the conserved domains C_1 -C_5 and has a deletion of at least one of the hypervariable domains V_1 -V_5.

2. The DNA sequence of claim 1 wherein said HIV-1 encoded mutein is strain SF2.

3. The DNA sequence of claim 1 in which at least hypervariable region V_1 is deleted from the encoded mutein.

4. The DNA sequence of claim 1 in which at least hypervariable region V_2 is deleted from the encoded mutein.

5. The DNA sequence of claim 1 in which at least hypervariable region V_3 is deleted from the encoded mutein.

6. The DNA sequence of claim 1 in which at least hypervariable region V_4 is deleted from the encoded mutein.

7. The DNA sequence of claim 1 in which at least hypervariable region V_5 is deleted from the encoded mutein.

8. The DNA sequence of claim 1 in which V_1 is deleted from the encoded mutein.

9. The DNA sequence of claim 1 in which V_2 is deleted from the encoded mutein.

10. The DNA sequence of claim 1 in which V_3 is deleted from the encoded mutein.

11. The DNA sequence of claim 1 in which V_4 is deleted from the encoded mutein.

12. The DNA sequence of claim 1 in which V_5 is deleted from the encoded mutein.

13. The DNA sequence of claim 1 in which V_1 and V_2 are deleted from the encoded mutein.

14. The DNA sequence of claim 1 in which V_3, V_4 and V_5 are deleted from the encoded mutein.

15. The DNA sequence of claim 1 in which V_1 through V_5 is deleted from the encoded mutein.

L1 ANSWER 5 OF 14 USPATFULL on STN
1998:118980 Immunoassay methods for the detection of HIV-1 antibodies employing envelope muteins containing hypervariable domain deletions.
Haigwood, Nancy L., Oakland, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 5814458 19980929
APPLICATION: US 1995-371618 19950112 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An immunoassay method of detecting antibodies to human deficiency virus type 1 (HIV-1), comprising: (a) providing a liquid sample to be tested for the presence of anti-HIV-1 antibodies; (b) contacting said sample with a human immunodeficiency virus type 1 (HIV-1) envelope mutein having the structure C_1 -V_1 -V_2 -C_2 -V_3 -C_3 -V_4 -C_4 -V_5 -C_5 wherein said mutein retains the conserved domains C_1 -C_5 and has a deletion of at least one of the hypervariable domains V_1 -V_5 ; and (c) detecting antibody bound specifically to said polypeptide.

2. The immunoassay of claim 1 in which said HIV-1 mutein is strain SF2.

3. The immunoassay of claim 1 in which at least hypervariable region V_1 is deleted from said mutein.

4. The immunoassay of claim 1 in which at least hypervariable region V_2 is deleted from said mutein.

5. The immunoassay of claim 1 in which at least hypervariable region V_3 is deleted from said mutein.

6. The immunoassay of claim 1 in which at least hypervariable region V_4 is deleted from said mutein.

7. The immunoassay of claim 1 in which at least hypervariable region V_5 is deleted from said mutein.

8. The immunoassay of claim 1 in which V_1 is deleted from said mutein.

9. The immunoassay of claim 1 in which V_2 is deleted from said mutein.

10. The immunoassay of claim 1 in which V_3 is deleted from said mutein.

11. The immunoassay of claim 1 in which V_4 is deleted from said mutein.

12. The immunoassay of claim 1 in which V_5 is deleted from said mutein.

13. The immunoassay of claim 1 in which V_1 and V_2 are deleted from said mutein.

14. The immunoassay of claim 1 in which V_3, V_4 and V_5 are deleted from said mutein.

15. The immunoassay of claim 1 in which V_1 through V_5 are deleted from said mutein.

16. The immunoassay of claim 1 in which the liquid sample is serum.

17. The immunoassay of claim 16 in which said serum is human serum.

L1 ANSWER 6 OF 14 USPATFULL on STN
1998:95237 HIV-1 envelope muteins lacking hypervariable domains.
Haigwood, Nancy L., Oakland, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 5792459 19980811
APPLICATION: US 1995-441184 19950515 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An isolated human immunodeficiency virus type 1 (HIV-1) envelope mutein having the structure C_1 --V_2 --C_2 --V_3 --C_3 --V_4 --C_4 --V_5 --C_5 wherein said mutein retains the conserved domains C_1 -C_5 and has a deletion of at least one of the hypervariable domains V_1 -V_5.

2. The mutein of claim 1 wherein said HIV-1 is strain SF2.

3. The mutein of claim 1 in which at least hypervariable region V_1 is deleted.

4. The mutein of claim 1 in which at least hypervariable region V_2 is deleted.

5. The mutein of claim 1 in which at least hypervariable region V_3 is deleted.

6. The mutein of claim 1 in which at least hypervariable region V_4 is deleted.

7. The mutein of claim 1 in which at least hypervariable region V_5 is deleted.

8. The mutein of claim 1 in which V_1 is deleted.

9. The mutein of claim 1 in which V_2 is deleted.

10. The mutein of claim 1 in which V_3 is deleted.

11. The mutein of claim 1 in which V_4 is deleted.

12. The mutein of claim 1 in which V_5 is deleted.

13. The mutein of claim 1 in which V1 and V2 are deleted.

14. The mutein of claim 1 in which V3, V4 and V5 are deleted.

15. The mutein of claim 1 in which V1 through V5 is deleted.

L1 ANSWER 7 OF 14 USPATFULL on STN
97:115395 Purified GP120 composition retaining natural conformation.
Haigwood, Nancy L., Oakland, CA, United States
Scandella, Carl, Oakland, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 5696238 19971209
APPLICATION: US 1995-439286 19950511 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method for purifying HIV gp120 so as to provide a purified gp120 glycopeptide having protein/protein binding properties substantially identical to natural viral HIV gp120, which comprises: a. fractionating a crude gp120 preparation containing full-length, glycosylated gp120 using ion exchange chromatography so as to provide a first collection of fractions; b. selecting a fraction from said first collection that exhibits specific binding affinity for CD4 peptide, thereby producing a first fractionated material; c. fractionating said first fractionated material by hydrophobic-interaction chromatography so as to provide a second collection of fractions; d. selecting a fraction from said second collection that exhibits specific binding affinity for CD4 peptide, thereby producing a second fractionated material; e. fractionating said second fractionated material by size exclusion chromatography so as to provide a third collection of fractions; and f. selecting a fraction from said third collection that exhibits specific binding affinity for CD4 peptide, thereby providing said purified gp120.

2. The method of claim 1, wherein said ion exchange chromatography occurs on a solid support having tertiary amine exchange groups.

3. The method of claim 2, wherein said solid support is diethylaminoethyl-substituted dextran.

4. The method of claim 3, wherein said chromatography is HPLC.

5. The method of claim 4, wherein separating in step (a) occurs at a pH of from 6 to 8.

6. The method of claim 1, wherein said hydrophobic interaction chromatography occurs on a solid support having pendant phenyl or aliphatic groups.

7. The method of claim 6, wherein said hydrophobic interaction chromatography occurs in two substep, a first substep in which the solid support is a phenyl agarose and a second substep in which the solid support is an aliphatic ether agarose.

8. The method of claim 7, wherein both substeps are HPLC.

9. The method of claim 8, wherein said separating in step (c) occurs using a decreasing ammonium sulfate gradient with an initial concentration of about 40% of saturation.

10. The method of claim 1, wherein said gel filtration chromatography uses a support capable of retarding molecules smaller than gp120.

11. The method of claim 10, wherein said gel filtration support has a fractionation range of about 50,000 to 200,000.

12. The method of claim 11, wherein said gel filtration chromatography, is HPLC.

13. The method of claim 1, further comprising the steps of: a. collecting a cell medium which contains a full-length, non-fusion, glycosylated gp120 protein, wherein said cell medium is a conditioned medium containing a non-HIV-infected cell that expresses said gp120; and b. concentrating said cell medium by removing molecules from said medium having molecular weights less than that of gp120, thereby producing a concentrated cell medium for use as said crude preparation.

14. The method of claim 1, wherein said second fractionated material is subjected to strong anion exchange chromatography prior to step (e).

15. The method of claim 14, wherein said strong anion exchange chromatography uses a solid support having quaternary ammonium exchange groups.

16. The method of claim 15, wherein said strong anion chromatography is carried out at a pH of from 7 to 9.

17. In a method of purifying full-length, glycosylated recombinant gp120, an improvement which comprises: purifying said gp120 as obtained from a cell culture medium to a purity of at least 95% as measured by SDS gel electrophoresis, wherein said purifying uses chromatography techniques selected from the group consisting of gel filtration, ion exchange, and hydrophobic interaction chromatography, with the proviso that no binding interaction between an antibody and said gp120 occurs at any time during said purifying.

18. The method of claim 17, with the further proviso that no contact between an organic solvent having a pH lower than about 4, or higher than about 9, and said gp120 occurs at any time during said purifying.

19. The method of claim 17, wherein said purifying comprises applying sequential steps of (1) cation exchange chromatography, (2) hydrophobic interaction chromatography, and (3) gel filtration to said cell culture medium.

20. The method of claim 19, wherein said steps are all HPLC steps.

L1 ANSWER 8 OF 14 USPATFULL on STN
97:106975 Vector for expression of a polypeptide in a mammalian cell.
Luciw, Paul A., Davis, CA, United States
Dina, Dino, San Francisco, CA, United States
Rosenberg, Steven, Oakland, CA, United States
Chapman, Barbara S., Berkeley, CA, United States
Thayer, Richard M., Alamo, CA, United States
Haigwood, Nancy L., Bellevue, WA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 5688688 19971118
APPLICATION: US 1994-288336 19940810 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A vector for expression of a polypeptide in a mammalian cell comprising a first polynucleotide sequence that comprises: a) an upstream SV40 origin of replication; b) a downstream SV40 polyadenylation region; and c) a transcription regulatory region from human cytomegalovirus immediate early region HCMV IE1, wherein the transcription regulatory region includes the first HCMV IE1 intron proximal to the 3' end of the HCMV IE1 promoter, is interposed between the SV40 origin of replication and the SV40 polyadenylation region, and is capable of directing the transcription of a polypeptide coding sequence operably linked downstream from the transcription regulatory region.

2. The vector of claim 1, wherein the polynucleotide sequence further comprises a linker that comprises a restriction site for insertion of the coding region of a polypeptide.

3. The vector of claim 2, wherein the restriction site is a SalI site.

4. The vector of claim 1, wherein the SV40 polyadenylation region comprises the SV40 polyadenylation sequence present in plasmid pSV7d.

5. The vector of claim 1, wherein the SV40 origin of replication comprises the SV40 origin of replication sequence present in plasmid pSVT2.

6. The vector of claim 1, further comprising a selectable marker.

7. The vector of claim 5, wherein the selectable marker is a polynucleotide sequence that encodes ampicillin resistance.

8. The vector of claim 1, further comprising a bacterial origin of replication.

9. The vector of claim 1, wherein the polynucleotide sequence comprises the HCMV sequences present in plasmid pCMV6ARV120tpa, ATCC Accession No. 68249.

10. The vector of claim 2, further comprising a coding region that encodes a polypeptide, inserted at the restriction site.

11. The vector of claim 10, further comprising a region encoding a signal sequence effective in directing the secretion of the polypeptide encoded by the coding region, positioned upstream from the coding region.

12. The vector of claim 11, wherein the signal sequence is derived from the human tissue plasminogen activator leader sequence.

13. A vector produced by the process comprising linking together in an operative manner: a) a SV40 origin of replication; b) a SV40 polyadenylation region; and c) a transcription regulatory region from human cytomegalovirus immediate early region HCMV IE1, wherein said regulatory region includes the first HCMV IE1 intron proximal to the 3' end of the HCMV IE1 promoter and is capable of directing the transcription of a polypeptide coding sequence operably linked downstream therefrom.

14. The vector of claim 13, wherein the vector is arranged in the same manner as plasmid pCMV6a.

15. A method for producing a vector for expression of a polypeptide in a mammalian cell comprising: a) providing a first polynucleotide molecule

that comprises a SV40 origin of replication; b) providing a second polynucleotide molecule that comprises a SV40 polyadenylation region; c) providing a third polynucleotide molecule that comprises a transcription regulatory region from human cytomegalovirus immediate early region HCMV IE1, wherein said regulatory region includes the first HCMV IE1 intron proximal to the 3' end of the HCMV IE1 promoter; and d) linking the SV40 origin of replication, the SV40 polyadenylation region and the regulatory region from HCMV IE1 together to form a vector that is capable of effecting the transcription of a polypeptide coding sequence operatively linked downstream from the regulatory region.

16. A method for producing the vector of claim 1, comprising introducing the vector into a host cell and allowing the host cell to generate a plurality of said vectors.

17. An isolated nucleic acid molecule comprising an enhanced promoter, wherein the enhanced promoter comprises the human cytomegalovirus immediate early region HCMV IE1 promoter and the first intron proximate to the 3' end of the HCMV IE1 promoter.

18. The nucleic acid molecule of claim 17, wherein the promoter region is derived from a subclone of human cytomegalovirus (Towne strain).

19. A vector for expression of a polypeptide in a mammalian cell, comprising the nucleic acid molecule of claims 17, wherein the nucleic acid molecule is capable of directing the transcription of a polypeptide coding sequence operably linked downstream of the nucleic acid molecule.

20. The vector of claim 19, further comprising an origin of replication operably linked upstream of the nucleic acid molecule.

21. The vector of claim 19, further comprising a polyadenylation region operably linked downstream of the nucleic acid molecule.

22. A vector for expression of a polypeptide in a mammalian cell, comprising: a) an upstream origin of replication; b) a downstream polyadenylation region; and c) the nucleic acid molecule of claim 17 interposed between the origin of replication and the polyadenylation region, wherein the enhanced promoter region is capable of directing the transcription of a polypeptide coding sequence operably linked downstream from the promoter region.

23. A method for constructing the vector of claim 19, comprising operatively linking together the nucleic acid molecule and the polypeptide coding sequence.

24. A method for producing the vector constructed in claim 23, comprising introducing the vector into a host cell that is capable of replicating the vector and allowing the host cell to replicate the vector.

L1 ANSWER 9 OF 14 USPATFULL on STN
97:70716 Peptide plasminogen activators.

Haigwood, Nancy L., Oakland, CA, United States
Mullenbach, Guy, Oakland, CA, United States
Afting, Ernst-Guenter, Marburg, Germany, Federal Republic of
Paques, Eric Paul, Marburg, Germany, Federal Republic of
Chiron Corp., Emeryville, CA, United States (U.S. corporation)Behringwerke AG, Marburg-Lahn, Germany, Federal Republic of (non-U.S. corporation)
US 5656269 19970812
APPLICATION: US 1995-381308 19950131 (8)

DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An isolated and purified human tissue plasminogen activator capable of dissolving clots and having at least one improved property selected from the group consisting of increased fibrin dependence and decreased plasminogen activator inhibitor susceptibility, said human tissue plasminogen activator having from about 3 to about 35 amino acids deleted from the C-terminus and wherein said deleted amino acids include Met-525 to Pro-527.

2. The human tissue plasminogen activator of claim 1 having amino acids Met-525 to Pro-527 deleted.

3. A pharmaceutical composition comprising a human tissue plasminogen activator according to claim 1 in an amount sufficient to provide dissolution of clots and a physiologically acceptable carrier.

4. The human tissue plasminogen activator of claim 1 having increased fibrin dependence.

5. The human tissue plasminogen activator of claim 1 having decreased plasminogen inhibitor susceptibility.

6. An isolated and purified nucleic acid encoding a human tissue plasminogen activator capable of dissolving clots and having at least one improved property selected from the group consisting of increased fibrin dependence and decreased plasminogen activator inhibitor susceptibility, said human tissue plasminogen activator having from about 3 to about 25 amino acids deleted from the C-terminus and wherein said deleted amino acids include Met-525 to Pro-527.

7. The nucleic acid of claim 6, wherein said nucleic acid is a deoxyribonucleic acid.

8. The nucleic acid of claim 6 encoding a human tissue plasminogen activator having amino acids Met-525 to Pro-527 deleted.

9. A vector comprising the nucleic acid set forth in claim 6.

10. A host cell expressing the human tissue plasminogen activator set forth in claim 1.

11. The host cell of claim 10, wherein said cell is expressing a human tissue plasminogen activator having amino acids Met-525 to Pro-527 deleted.

12. A host cell having the nucleic acid set forth in claim 6.

13. A method of producing a human tissue plasminogen activator comprising the steps: providing the host cell of claim 10 and culturing said cell under conditions wherein a human tissue plasminogen activator is produced.

14. A method of producing a human tissue plasminogen activator comprising the steps: providing the host cell of claim 11 and culturing said cell under conditions wherein a human tissue plasminogen activator is produced.

L1 ANSWER 10 OF 14 USPATFULL on STN
97:68163 Purified gp120 composition retaining natural conformation.

Haigwood, Nancy L., Oakland, CA, United States
Scandella, Carl, Oakland, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 5653985 19970805
APPLICATION: US 1995-439119 19950511 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for stimulating the formation of antibodies capable of neutralizing infection by an HIV viral isolate in at least one mammalian species, which comprises immunizing a mammalian subject with an antigenic composition containing purified, full-length, non-fusion recombinant HIV gp120 glycoprotein having protein/protein-interaction properties substantially identical to gp120 as presented on an HIV virus.

2. The method of claim 1, wherein said mammal is a primate.

3. The method of claim 1 wherein the protein/protein-interaction properties of the glycoprotein comprise: (a) binding affinity for CD4; (b) binding affinity for an antibody capable of neutralizing HIV infectivity; or (c) binding affinity for gp41.

4. The method of claim 1 wherein the composition further comprises an adjuvant.

5. The method of claim 4 wherein the adjuvant is an aluminum salt.

6. The method of claim 4 wherein the adjuvant is an oil-in-water emulsion formed from a metabolizable oil and an emulsifying agent.

7. The method of claim 1 wherein the composition comprises at least about 1 mg of the glycoprotein.

8. The method of claim 7 wherein the composition is suspended in a volume of a pharmaceutical vehicle or carrier.

9. The method of claim 8 wherein the volume of the pharmaceutical vehicle or carrier is about 0.1 to 1.0 mL.

10. The method of claim 9 wherein the composition is administered to the mammalian subject by injection.

L1 ANSWER 11 OF 14 USPATFULL on STN
97:25124 Purified gp120 compositions retaining natural conformation.
Haigwood, Nancy L., 7050 Sayer Dr., Oakland, CA, United States 94611
Scandella, Carl, 7050 Sayer Dr., Oakland, CA, United States 94611
US 5614612 19970325
APPLICATION: US 1994-240073 19940509 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A purified, full-length, non-fusion recombinant HIV gp120 glycoprotein having protein/protein-interaction properties substantially identical to gp120 as presented on an HIV virus and being substantially free of other proteins.

2. The glycoprotein of claim 1, wherein said properties comprise: a. binding affinity for CD4; b. binding affinity for an antibody capable of neutralizing HIV infectivity; or c. binding affinity for gp41.

3. The glycoprotein of claim 2, wherein binding affinity for CD4 is measured by determining the fraction of gp120 that binds to CD4 using a gel filtration assay.

4. The glycoprotein of claim 3, wherein said antibody is a chimpanzee or human antibody.

5. The glycoprotein of claim 1, wherein said glycoprotein is prepared by a process comprising: a. fractionating a crude gp120 preparation containing full-length, glycosylated gp120 using ion exchange chromatography so as to provide a first collection of fractions; b. selecting a fraction from said first collection that exhibits specific binding affinity for CD4 peptide, thereby producing a first fractionated material; c. fractionating said first fractionated material by hydrophobic-interaction chromatography so as to provide a second collection of fractions; d. selecting a fraction from said second collection that exhibits specific binding affinity for CD4 peptide, thereby producing a second fractionated material; e. fractionating said second fractionated material by size exclusion chromatography so as to provide a third collection of fractions; and f. selecting a fraction from said third collection that exhibits specific binding affinity for CD4 peptide, thereby providing said purified gp120.

6. The glycoprotein of claim 3, having a binding affinity (Kd) for CD4 of about 6.9 nM as measured using a gel filtration assay.

7. The glycoprotein of claim 5, wherein said protein/protein-interaction properties comprise: a) binding affinity for CD4; b) binding affinity for an antibody capable of neutralizing HIV infectivity; or c) binding affinity for gp41.

8. The glycoprotein of claim 7, wherein binding affinity for CD4 is measured by determining the fraction of gp120 that binds to CD4 using a gel filtration assay.

9. The glycoprotein of claim 8, having a binding affinity (Kd) for CD4 of about 6.9 nM as measured using a gel filtration assay.

10. The glycoprotein of claim 7, wherein said antibody is a chimpanzee or human antibody.

L1 ANSWER 12 OF 14 USPATFULL on STN
96:24742 Peptide plasminogen activators.
Haigwood, Nancy L., Oakland, CA, United States
Afting, Ernest-Guenter, Marburg, Germany, Federal Republic of
Mullenbach, Guy, Oakland, CA, United States
Paques, Eric P., Marburg, Germany, Federal Republic of
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)Behringwerke AG, Marburg, Germany, Federal Republic of (non-U.S. corporation)
US 5501853 19960326
APPLICATION: US 1986-944117 19861222 (6)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A human tissue plasminogen activator capable of dissolving clots and having at least one improved property selected from the group consisting of improved plasminogenolytic specific activity, increased fibrin dependence, and decreased plasminogen inhibitor susceptibility, said human tissue plasminogen activator having the lysine at amino acid position 277 substituted with arginine and having from 3 to 25 amino

acids deleted from the C-terminus.

2. A human tissue plasminogen activator capable of dissolving clots and having at least one improved property selected from the group consisting of improved plasminogenolytic specific activity, increased fibrin dependence, and decreased plasminogen inhibitor susceptibility, said human tissue plasminogen activator having from 3 to 25 amino acids deleted from the C-terminus and having at least one of the group consisting of asparagine 117, asparagine 184 and asparagine 448 substituted with glutamine.

3. A human tissue plasminogen activator capable of dissolving clots and having at least one improved property selected from the group consisting of improved plasminogenolytic specific activity, increased fibrin dependence, and decreased plasminogen inhibitor susceptibility, said human tissue plasminogen activator having the lysine at amino acid position 277 substituted with arginine and having at least one of the group consisting of asparagine 117, asparagine 184 and asparagine 448 substituted with glutamine.

4. A human tissue plasminogen activator capable of dissolving clots and having at least one improved property selected from the group consisting of improved plasminogenolytic specific activity, increased fibrin dependence, and decreased plasminogen inhibitor susceptibility, said human tissue plasminogen activator having the lysine at amino acid position 277 substituted with arginine, having from 3 to 25 amino acids deleted from the C-terminus and having at least one of the group consisting of asparagine 117, asparagine 184 and asparagine 448 substituted with glutamine.

5. The human tissue plasminogen activator of any one of claims 1, 2 or 4 having Met-525 to Pro-527 deleted.

6. The human tissue plasminogen activator of any one of claims 2, 3 or 4, having at least two of the group consisting of asparagine 117, asparagine 184 and asparagine 448 substituted with glutamine.

7. The human tissue plasminogen activator of either of claim 3 or claim 4 having each of asparagine 117, asparagine 184 and asparagine 448 substituted with glutamine.

8. A nucleic acid encoding a human tissue plasminogen activator as set forth in any one of claims 1, 2, 3, or 4.

9. A recombinant vector encoding a human tissue plasminogen activator as set forth in any one of claims 1, 2, 3, or 4.

10. A host cell expressing a human tissue plasminogen activator as set forth in any one of claims 1, 2, 3, or 4.

11. A process for producing a human tissue plasminogen activator comprising: providing a host cell capable of expressing a human tissue plasminogen activator as set forth in any one of claims 1, 2, 3, or 4; culturing said cell under conditions wherein said human tissue plasminogen activator is produced; and recovering the human tissue plasminogen activator produced.

12. A pharmaceutical composition comprising a human tissue plasminogen activator according to any one of claims 1, 2, 3, or 4 in an amount sufficient to provide for dissolution of clots and a physiologically acceptable carrier.

13. The human tissue plasminogen activator of either of claim 3 or claim 4 having each of asparagine 117, asparagine 184 and asparagine 448 substituted with glutamine and having Met-525 to Pro-527 deleted.

14. A nucleic acid encoding a human tissue plasminogen activator as set forth in claim 13.

15. A pharmaceutical composition comprising a human tissue plasminogen activator according to claim 13 in an amount sufficient to provide for dissolution of clots and a physiologically acceptable carrier.

L1 ANSWER 13 OF 14 USPATFULL on STN
95:71135 Peptide plasminogen activators.
Afting, Ernest-Guenter, Marburg, Germany, Federal Republic of
Paques, Eric-Paul, Marburg, Germany, Federal Republic of
Haigwood, Nancy L., Oakland, CA, United States
Mullenbach, Guy, Oakland, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)Behringwerke AG, Marburg-Lahn, Germany, Federal Republic of (non-U.S. corporation)
US 5439679 19950808
APPLICATION: US 1994-208202 19940310 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A human tissue plasminogen activator capable of dissolving clots and having at least one improved property selected from the group consisting of improved plasminogenolytic specific activity, increased fibrin dependence, and decreased plasminogen inhibitor susceptibility, said human tissue plasminogen activator having a lysine at amino acid position 277 substituted with arginine.

2. A host cell expressing the human tissue plasminogen activator set forth in claim 1.

3. The host cell of claim 2, wherein said cell is a mammalian cell.

4. The host cell of claim 3, wherein said cell is selected from the group consisting of CHO and COS cells.

5. A method of producing a human tissue plasminogen activator comprising the steps: providing the host cell of claim 2 and culturing said cell under conditions wherein a human tissue plasminogen activator is produced.

6. A pharmaceutical composition comprising a human tissue plasminogen activator according to claim 1 in an amount sufficient to provide for dissolution of clots and a physiologically acceptable carrier.

7. The human tissue plasminogen activator according to claim 1 having decreased plasminogen inhibitor susceptibility.

8. A nucleic acid encoding a human tissue plasminogen activator capable of dissolving clots and having at least one improved property selected from the group consisting of improved plasminogenolytic specific activity, increased fibrin dependence, and decreased plasminogen inhibitor susceptibility, said human tissue plasminogen activator having a lysine at amino acid position 277 substituted with arginine.

9. The nucleic acid of claim 8, wherein said nucleic acid is a deoxyribonucleic acid.

10. A recombinant vector comprising the nucleic acid of claim 9.

11. The recombinant vector of claim 10, wherein said vector is an expression vector.

12. A host cell having the recombinant vector set forth in claim 11.

13. A host cell having the nucleic acid set forth in claim 8.

L1 ANSWER 14 OF 14 USPATFULL on STN
92:34068 Expression of TPA in mammalian cells.
Haigwood, Nancy L., Oakland, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 5108909 19920428
APPLICATION: US 1987-97271 19870917 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A DNA construct, comprising: a chimeric gene having a coding sequence for human tissue plasminogen activator with at least one intron of at least 100 bp at a site natural to the genomic form of human tissue plasminogen activator gene, wherein said intron includes a 5' splice site and a 3' splice site and provides increased expression of said chimeric gene relative to an uninterrupted coding sequence lacking all introns and wherein said chimeric gene is capable of producing mRNA having the proper reading frame wherein said gene contains fewer than the total number of introns present in the genomic form of the gene for human tissue plasminogen activator, and transcriptional initiation and termination regulatory sequences functional in a mammalian host, at least the initiation sequence being an initiation sequence other than the initiation sequence for the genomic form of the gene for human tissue plasminogen activator.

2. An expression vector capable of stable maintenance in a mammalian host comprising a DNA construct according to claim 1.

3. A mammalian cell in culture containing an expression vector according to claim 2.

4. A mammalian cell in culture wherein DNA construct according to claim 1 is integrated into a chromosome.

5. A DNA construct according to claim 1, wherein each of said introns is natural to human tissue plasminogen activator gene.

6. An expression vector capable of stable maintenance in a mammalian host comprising a DNA construct according to claim 5.

7. A DNA construct according to claim 5, wherein a 2.6 Kb EcoRI-EcoRI fragment from complete digestion with EcoRI of the genomic gene of human tissue plasminogen activator is substituted for an EcoRI-EcoRI region of a cDNA coding for human tissue plasminogen activator.

8. A mammalian cell in culture wherein a DNA construct according to claim 7 is integrated into a chromosome.

9. An expression vector capable of stable maintenance in a mammalian host comprising a DNA construct according to claim 7.

10. A mammalian cell in culture containing an expression vector

according to claim 9.

11. A method for producing tissue plasminogen activator in a mammalian cell host comprising: providing a chimeric gene for human tissue plasminogen activator with at least one intron of at least 100 bp at a site natural to the genomic form of human tissue plasminogen activator gene, wherein said intron includes a 5' splice site and a 3' splice site and provides increased expression of said chimeric gene relative to an uninterrupted coding sequence lacking all introns and wherein said chimeric gene is capable of producing mRNA having the proper reading frame, wherein said gene contains fewer than the total number of introns present in the genomic form of the gene for human tissue plasminogen activator; placing said chimeric gene for human tissue plasminogen activator with at least one intron under the transcriptional control of a promoter region other than the promoter region natural to human tissue plasminogen activator gene; introducing said chimeric gene for human tissue plasminogen activator with at least one intron under control of said promoter region into said mammalian cell host; culturing said mammalian cell host; and expressing said chimeric gene for human tissue plasminogen activator with at lest one intron in said mammalian cell host.

12. A method according to claim 11, wherein said chimeric gene comprises cDNA and genomic DNA having at least one intron.

13. A DNA construct according to claim 12, wherein a 2.6 Kb EcoRI-EcoRI fragment from complete digestion with EcoRI of the genomic gene of human tissue plasminogen activator is substituted for an EcoRI-EcoRI region of a cDNA coding for human tissue plasminogen activator.

14. A method according to claim 4, wherein the introns are natural to human tissue plasminogen activator gene.

15. A method according claim 14, having a viral promoter region for regulating transcription of said gene.

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http://www.stn-international.de/stndatabases/details/dwpi_r.html <<<

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E2           1      HAIGWOOD N/IN
E3          12 --> HAIGWOOD N L/IN
E4           1      HAIHONG Z/IN
E5           1      HAIISAGUERRE M/IN
E6           1      HAIJEMA B J/IN
E7           1      HAIJI H/IN
E8           1      HAIJIE Z/IN
E9          10      HAIJIMA A/IN
E10          1      HAIJIMA E/IN
E11          4      HAIJIMA H/IN
E12          3      HAIJIMA K/IN

=> s e3
L2          12 "HAIGWOOD N L"/IN

=> d l2,bib,ab,1-12

L2   ANSWER 1 OF 12  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   2005-796080 [81]   WPIDS
DNC  C2005-245261
TI   A vaccination protocol comprises administering a first immunogen
     comprising Human Immunodeficiency Virus type 1 Envelope (env) sequence,
     and a second immunogen comprising a primate immunodeficiency virus env
     sequence.
DC   B04 D16
IN   BLAY, W; HAIGWOOD, N L; STAMATATOS, L
PA   (BLAY-I) BLAY W; (HAIG-I) HAIGWOOD N L; (STAM-I) STAMATATOS L
CYC  1
PI   US 2005260235   A1 20051124 (200581)*      21
ADT  US 2005260235 A1 Provisional US 2004-558181P 20040331, US 2005-96698
     20050331
PRAI US 2004-558181P      20040331; US 2005-96698        20050331
AB   US2005260235 A UPAB: 20051216
     NOVELTY - A vaccination protocol comprising administering to a primate
     host a first immunogen comprising at least one Human Immunodeficiency
     Virus type 1 Envelope (env) sequence having a first set of consensus
     glycosylation sequences, followed by a second immunogen comprising at
     least one primate immunodeficiency virus env sequence having a second set
     of consensus glycosylation sequences, is new.
          DETAILED DESCRIPTION - A vaccination protocol comprising
     administering to a primate host a first immunogen comprising at least one
     Human Immunodeficiency Virus type 1 Envelope (env) sequence having a first
     set of consensus glycosylation sequences, followed by a second immunogen
     comprising at least one primate immunodeficiency virus env sequence having
     a second set of consensus glycosylation sequences, where the differences
     between the first set of consensus glycosylation sequences and the second
     set of consensus glycosylation sequences comprise at least one of:
          (a) an addition of a consensus N-linked glycosylation sequence in V1
     at a position corresponding to position 143 or 144 in the HIV-89.6 env
     sequence;
          (b) a shift of a consensus N-linked glycosylation sequence in V2 from
     a position corresponding to position 186 in the HIV-89.6 env sequence to a
     position corresponding to position 188 in the HIV-89.6 env sequence;
          (c) an addition of a consensus N-linked glycosylation sequence
     addition in C2 at a position corresponding to position 276 in the HIV-89.6
     env sequence;
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(d) an addition of a consensus N-linked glycosylation sequence addition in V4 at a position corresponding to position 386 in the HIV-89.6 env sequence;

(e) an addition of a consensus N-linked glycosylation sequence addition in C2 at a position corresponding to position 397 in the HIV-89.6 env sequence; or

(f) a shift of a consensus N-linked glycosylation sequence in V5 from a position corresponding to position 460 in the HIV-89.6 env sequence to a position corresponding to position 462 or 463 in the HIV-89.6 env sequence.

ACTIVITY - None given.

MECHANISM OF ACTION - Vaccine.

USE - The vaccination protocol is useful as therapeutic vaccines in primate hosts that are already infected with one or more primate immunodeficiency virus. It can be used for administering immunogens to a primate host in order to promote the formation of neutralizing antibodies against primate immunodeficiency virus.

Dwg.0/6

L2 ANSWER 2 OF 12 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2005-371628 [38] WPIDS
DNC C2006-151777
TI New vaccination protocol against HIV infection comprises immunizing a host with a first HIV envelope immunogen followed by booster immunizations with a second HIV envelope immunogen having more glycosylation sites than the first immunogen.
DC B04 D16
IN **HAIGWOOD, N L**
PA (HAIG-I) HAIGWOOD N L
CYC 1
PI US 2005112138 A1 20050526 (200538)* 13
ADT US 2005112138 A1 US 2003-719004 20031121
PRAI US 2003-719004 20031121
AB US2005112138 A UPAB: 20060804

NOVELTY - A vaccination protocol comprising immunizing a mammalian host with a first HIV envelope immunogen having a minimum number of N- and O-linked glycosylation sites, followed by one or more booster immunizations with at least one second HIV envelope immunogen having more glycosylation sites than the first immunogen, is new.

ACTIVITY - Anti-HIV.

No biological data given.

MECHANISM OF ACTION - Vaccine.

USE - The vaccination protocol is useful for generating HIV neutralizing antibodies or for preventing HIV/AIDS infections.

Dwg.0/5

L2 ANSWER 3 OF 12 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2000-037079 [03] WPIDS
CR 1996-179247 [18]; 1997-414546 [38]
DNC C2000-009446
TI A human tissue plasminogen activator.
DC B04 D16
IN AFTING, E; **HAIGWOOD, N L**; MULLENBACH, G; PAQUES, E P
PA (CHIR) CHIRON CORP; (HMRI) HOECHST MARION ROUSSEL DEUT GMBH
CYC 1
PI US 5976530 A 19991102 (200003)* 9
ADT US 5976530 A CIP of US 1985-812879 19851223, Cont of US 1986-944117 19861222, Cont of US 1995-381308 19950131, US 1997-799823 19970213
FDT US 5976530 A Cont of US 5501853, Cont of US 5656269
PRAI US 1986-944117 19861222; US 1985-812879 19851223;

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     US 1995-381308       19950131; US 1997-799823       19970213
AB   US    5976530 A UPAB: 20000118
     NOVELTY - A human tissue plasminogen activator (tPA; I) is capable of
     dissolving clots. (I) has lysine 277 substituted with another amino acid,
     and further comprises a deletion of 3-25 amino acids from the C-terminus.
          DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:
          (1) a pharmaceutical composition comprising (I);
          (2) a nucleic acid molecule encoding (I);
          (3) a vector comprising the nucleic acid sequence of (2);
          (4) a preparation of the vector of (3) comprising isolating the
     nucleic acid sequence and inserting it into an expression vector such that
     it can be expressed in a host cell;
          (5) a host cell comprising the nucleic acid sequence of (2); and
          (6) a preparation of (I), comprising culturing a host cell comprising
     a nucleic acid encoding (I) and isolating the protein.
          USE - (I) is used to dissolve clots.
          ADVANTAGE - Substantial advantages can be achieved by making changes
     in the wild-type tPA amino acid sequence. Not only can activity be
     increased, but at the same time sensitivity to plasminogen activator
     inhibitor can be decreased, so that overall a very substantial increase in
     effective activity can be achieved in vivo. Also, the enzyme can be made
     substantially more specific in providing for enhanced fibrin dependence,
     so that it has substantially reduced activity in the absence of clots.
     Thus, one can administer lower amounts of these polypeptides with
     plasminogen activator activity, so as to minimize the level in the blood
     stream of the protein (or enzyme) and substantially diminish the
     undesirable side effects of tPA, while for increased activity against
     clots.
     Dwg.0/0

L2   ANSWER 4 OF 12  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   1998-041353 [04]   WPIDS
CR   1991-295578 [40];  1997-201533 [18];  1997-414049 [37]
DNC  C1998-013780
TI   Purification of HIV gp120 - using chromatographic methods.
DC   B04 D16
IN   HAIGWOOD, N L; SCANDELLA, C
PA   (CHIR) CHIRON CORP
CYC  1
PI   US 5696238      A 19971209 (199804)*      53
ADT  US 5696238 A Cont of US 1991-684963 19910820, Cont of US 1993-109002
     19930816, Div ex US 1994-240073 19940509, US 1995-439286 19950511
FDT  US 5696238 A Div ex US 5614612
PRAI US 1991-684963       19910820; US 1993-109002       19930816;
     US 1994-240073       19940509; US 1995-439286       19950511
AB   US    5696238 A UPAB: 19980126
     A novel method for purifying HIV gp120 so as to provide a purified gp120
     glycopeptide having protein/protein binding properties substantially
     identical to natural viral HIV gp120, comprises: (a) fractionating a crude
     gp120 preparation containing full-length, glycosylated gp120 using ion
     exchange chromatography so as to provide a first collection of fractions;
     (b) selecting a fraction from the first collection that exhibits specific
     binding affinity for CD4 peptide, thereby producing a first fractionated
     material; (c) fractionating the first fractionated material by hydrophobic
     interaction chromatography so as to provide a second collection of
     fractions; (d) selecting a fraction from the second collection that
     exhibits specific binding affinity for CD4 peptide, thereby producing a
     second fractionated material; (e) fractionating the second fractionated
     material by size exclusion chromatography so as to provide a third
     collection of fractions; and (f) selecting a fraction from the third
     collection that exhibits specific binding affinity for CD4 peptide,
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thereby providing the purified gp120.
USE - The purified gp120 can be used for antibody production and in vaccines.
Dwg.0/7

L2 ANSWER 5 OF 12 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 1998-007982 [01] WPIDS
CR 1986-126568 [20]; 1992-373025 [45]; 1992-417329 [51]; 2000-170256 [15]; 2002-742717 [81]; 2003-182063 [18]; 2003-511942 [48]
DNC C1998-002743
TI Enhanced promoter for gene expression - comprising cytomegalovirus immediate early promoter plus intron.
DC B04 D16
IN CHAPMAN, B S; DINA, D; **HAIGWOOD, N L**; LUCIW, P A; ROSENBERG, S; THAYER, R M
PA (CHIR) CHIRON CORP
CYC 1
PI US 5688688 A 19971118 (199801)* 99
ADT US 5688688 A CIP of US 1984-667501 19841031, CIP of US 1985-696534 19850130, CIP of US 1985-773447 19850906, Div ex US 1987-138894 19871224, Cont of US 1992-931191 19920817, Div ex US 1993-83391 19930628, Div ex US 1993-107377 19930817, US 1994-288336 19940810
FDT US 5688688 A Div ex US 5156949
PRAI US 1987-138894 19871224; US 1984-667501 19841031;
US 1985-696534 19850130; US 1985-773447 19850906;
US 1992-931191 19920817; US 1993-83391 19930628;
US 1993-107377 19930817; US 1994-288336 19940810
AB US 5688688 A UPAB: 20030729
An isolated nucleic acid molecule comprising an enhanced promoter is claimed, where the enhanced promoter comprises the human cytomegalovirus immediate early region (HCMV IE1) promoter and the first intron proximate to the 3' end of the HCMV IE1 promoter.
USE/ADVANTAGE - Expression of HIV gp120 by COS 7 cells transfected with pCMV6a containing the gp120 coding region, where pCMV6a is a vector containing the above enhanced promoter, is increased by a factor of 50-100 compared with the use of a vector containing the SV40 early promoter.
Dwg.29/31

L2 ANSWER 6 OF 12 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 1997-414546 [38] WPIDS
CR 1996-179247 [18]; 2000-037079 [03]
DNC C1997-132633
TI Human tissue plasminogen activator deletion mutant - with increased fibrin dependence and decreased susceptibility to plasminogen activator inhibitor.
DC B04 D16
IN AFTING, E; **HAIGWOOD, N L**; MULLENBACH, G; PAQUES, E P
PA (BEHW) BEHRINGWERKE AG; (CHIR) CHIRON CORP
CYC 1
PI US 5656269 A 19970812 (199738)* 9
ADT US 5656269 A CIP of US 1985-812879 19851223, Cont of US 1986-944117 19861222, US 1995-381308 19950131
FDT US 5656269 A Cont of US 5501853
PRAI US 1986-944117 19861222; US 1985-812879 19851223;
US 1995-381308 19950131
AB US 5656269 A UPAB: 20000118
New human tissue plasminogen activator (tPA) mutant (I) has 3-35 amino acids deleted from the C terminus, including Met-525 to Pro-527, provided that it is capable of dissolving clots and has increased fibrin dependence and/or decreased susceptibility to plasminogen activator inhibitor

(compared with wild-type tPA). Also claimed are: (1) a nucleic acid encoding (I), (2) a vector containing the nucleic acid, and (3) a host cell expressing (I) or a host cell having the nucleic acid.

USE - The tPA deletion mutants are useful in the lysis of fibrin clots and prevention of blood clot formation.

ADVANTAGE - Decreased PAI susceptibility increases effective activity in vivo and increased fibrin dependence reduces activity in the absence of clots, allowing lower doses to be used and reducing side effects.

Dwg.0/0

L2 ANSWER 7 OF 12 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 1997-414049 [38] WPIDS

CR 1991-295578 [40]; 1997-201533 [18]; 1998-041353 [04]

DNC C1997-132499

TI Inducing neutralising antibodies against human immunodeficiency virus - using non-fusion recombinant gp120 as antigen, used for treatment or prevention of infection.

DC B04 D16

IN **HAIGWOOD, N L**; SCANDELLA, C

PA (CHIR) CHIRON CORP

CYC 1

PI US 5653985 A 19970805 (199738)* 52

ADT US 5653985 A CIP of US 1990-490858 19900309, Cont of US 1991-684963 19910820, Cont of US 1993-109002 19930816, Div ex US 1994-240073 19940509, US 1995-439119 19950511

PRAI US 1991-684963 19910820; US 1990-490858 19900309;
US 1993-109002 19930816; US 1994-240073 19940509;
US 1995-439119 19950511

AB US 5653985 A UPAB: 19980126

The formation of antibodies (Ab) able to neutralise infection by a human immunodeficiency virus (HIV) isolate in one or more mammalian species is stimulated by immunisation with an antigenic composition (A) containing purified, full-length, non-fusion recombinant HIV gp120 glycoprotein (I). (I) has protein-protein interaction properties identical to those of the native gp120 presented on HIV.

USE - (A) are useful in vaccines for the prevention and treatment of HIV-1 infection. They can also be used to assay anti-HIV antibodies; to generate anti-HIV serum and as assay standards.

ADVANTAGE - (I) has binding and other immunological properties practically the same as native gp120. It is produced by a process that does not involve denaturation or exposure to harsh solvent conditions and can induce neutralising antibodies against many different isolates. (I) is cheaper to produce and safer than existing vaccines.

Dwg.1/7

L2 ANSWER 8 OF 12 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 1997-201533 [18] WPIDS

CR 1991-295578 [40]; 1997-414049 [37]; 1998-041353 [04]

DNC C1997-064438

TI Purified recombinant human immunodeficiency virus glycoprotein 120 - useful in immunoassays and for antiserum or vaccine production.

DC B04 D16

IN **HAIGWOOD, N L**; SCANDELLA, C

PA (HAIG-I) HAIGWOOD N L; (SCAN-I) SCANDELLA C

CYC 1

PI US 5614612 A 19970325 (199718)* 52

ADT US 5614612 A CIP of US 1990-490858 19900309, CIP of WO 1991-US1484 19910308, Cont of US 1991-684963 19910820, Cont of US 1993-109002 19930816, US 1994-240073 19940509

PRAI US 1991-684963 19910820; US 1990-490858 19900309;

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     WO 1991-US1484        19910308; US 1993-109002        19930816;
     US 1994-240073        19940509
AB   US    5614612 A UPAB: 19980126
     Purified, full-length, non-fusion recombinant human immunodeficiency virus
     (HIV) glycoprotein (gp) 120 having the same protein/protein interaction
     properties as gp120 presented on HIV, which is free of other proteins, is
     claimed.
          USE - The recombinant gp120 can be used in immunoassays for anti-HIV
     antibodies, in anti-HIV antiserum production and in vaccines.
          ADVANTAGE - The recombinant gp120 can be obtained in a form that has
     never been denatured or subjected to harsh solvent conditions, e.g.
     affinity chromatography conditions, so that it retains the CD4 receptor
     binding properties of natural HIV gp120, unlike prior art isolated forms
     of gp120.
     Dwg.0/7

L2   ANSWER 9 OF 12  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   1996-179247 [18]   WPIDS
CR   1997-414546 [38]; 2000-037079 [03]
DNC  C1996-056474
TI   Human tissue plasminogen activator mutants for fibrin clot lysis  - with
     improved plasminogenolytic specific activity, increased fibrin dependence
     and decreased plasminogen inhibitor susceptibility.
DC   B04 D16
IN   AFTING, E; HAIGWOOD, N L; MULLENBACH, G; PAQUES, E P
PA   (BEHW) BEHRINGWERKE AG; (CHIR) CHIRON CORP
CYC  1
PI   US 5501853       A  19960326 (199618)*       8
ADT  US 5501853 A CIP of US 1985-812879 19851223, US 1986-944117 19861222
PRAI US 1986-944117        19861222; US 1985-812879        19851223
AB   US    5501853 A UPAB: 20000118
     Novel human tissue plasminogen activator (tPA) capable of dissolving
     clots, with at least 1of the following improved properties: (a) improved
     plasminogenolytic specific activity; (b) increased fibrin dependence; and
     (c) decreased plasminogen inhibitor susceptibility, has 3-25 amino acids
     opt. deleted from its C-terminus, and Lys 277 substd. with Arg. and/or at
     least 1 of Asn 117, 184 or 448 substd. with Gln. Also claimed are: (1)
     nucleic acid encoding the above tPA; (2) recombinant vector encoding the
     above tPA; and (3) host cell expressing the above tPA.
          USE - The tPA can be used in the lysis of fibrin clots (claimed), and
     in the prevention of blood clot formation by activating plasminogen
          ADVANTAGE - The specific activity of tPA is enhanced by reducing the
     amount of glycosylation in the mol., e.g. by modifying the glycosylation
     sites at positions 117-119, 184-186 and/or 448-450, the sensitivity of tPA
     to plasminogen activator inhibition is reduced by modifying the cleavage
     site which occurs at the amino acids 274-278 and the fibrin dependence of
     tPA is increased by truncating the C-terminus.
     Dwg.0/0

L2   ANSWER 10 OF 12  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   1991-295578 [40]   WPIDS
CR   1997-201533 [18]; 1997-414049 [38]; 1998-041353 [04]
DNC  C1991-127781
TI   Purificn. of HIV glyco-protein gp120 - for use as a vaccine against HIV-1
     infections.
DC   B04 C03 D16
IN   HAIGWOOD, N L; SCANDELLA, C; SCANDELLA, C J
PA   (CHIR) CHIRON CORP
CYC  18
PI   WO 9113906       A  19910919 (199140)*
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      RW: AT BE CH DE DK ES FR GB GR IT LU NL SE
       W: CA JP
     PT 96994        A  19911031 (199148)
     EP 519001       A1 19921223 (199252)  EN  109
        R: AT BE CH DE FR GB IT LI LU NL SE
     JP 05505616     W  19930819 (199338)      30
     JP 2624894      B2 19970625 (199730)      47
     JP 09227588     A  19970902 (199745)      46
     CA 2077753      C  20000229 (200030)  EN
     EP 519001       B1 20011031 (200169)  EN
        R: AT BE CH DE FR GB IT LI LU NL SE
     DE 69132795     E  20011213 (200205)
     IE 83584        B  20040922 (200462)
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ADT EP 519001 A1 EP 1991-906615 19910308, WO 1991-US1484 19910308; JP 05505616 W JP 1991-507168 19910308, WO 1991-US1484 19910308; JP 2624894 B2 JP 1991-507168 19910308, WO 1991-US1484 19910308; JP 09227588 A Div ex JP 1991-507168 19910308, JP 1996-192595 19910308; CA 2077753 C CA 1991-2077753 19910308, WO 1991-US1484 19910308; EP 519001 B1 EP 1991-906615 19910308, WO 1991-US1484 19910308; DE 69132795 E DE 1991-632795 19910308, EP 1991-906615 19910308, WO 1991-US1484 19910308; IE 83584 B IE 1991-779 19910308

FDT EP 519001 A1 Based on WO 9113906; JP 05505616 W Based on WO 9113906; JP 2624894 B2 Previous Publ. JP 05505616, Based on WO 9113906; CA 2077753 C Based on WO 9113906; EP 519001 B1 Based on WO 9113906; DE 69132795 E Based on EP 519001, Based on WO 9113906

PRAI US 1990-490858 19900309

AB WO 9113906 A UPAB: 20040928
Purificn. HIV gp. 120 is claimed, which gives the glycoprotein with protein/protein binding properties identical to those of natural gp. 120. The method comprises: (a) fractionating a crude gp. 120 prepn. by ion exchange chromatography to give a collection of fractions; (b) selecting a fraction exhibiting specific binding affinity for CD4 peptide; (c) fractionating this fraction by hydrophobic-interaction chromatography; (d) selecting the resulting fraction exhibiting specific binding affinity for CD4 peptide; (e) fractionating this by size exclusion chromatography; and (f) selecting the fraction exhibiting specific binding affinity for CD4 peptide, giving pure gp. 120.

Also claimed are pure, full-length, non-fusion recombinant HIV gp. 120, a method of forming antibodies which neutralised an HIV viral isolate and a method of purifying full-length glycosylated recombinant gp. 120.

USE/ADVANTAGE - The gp. 120 is used as a vaccine and to treat HIV-1 infection. Vaccine dosages are 0.1-250 mg/dose. Admin. is oral or by injection. @(109pp Dwg.No.0/7)

L2 ANSWER 11 OF 12 WPIDS

Full Text

AN 1990-115824 [15] WPIDS

DNC C1990-050863

TI HIV-1 envelope mutein lacking hyper-variable domains - used for diagnosis of antibodies, antibody prodn. and for prodn. of vaccines.

DC B04 D16

IN **HAIGWOOD, N L**

PA (CHIR) CHIRON CORP

CYC 13

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PI   WO 9002568      A  19900322 (199015)*     110
      RW: AT BE CH DE FR GB IT LU NL SE
       W: JP
     EP 434713       A  19910703 (199127)
        R: AT BE CH DE FR GB IT LU NL SE
     EP 434713       B1 19941012 (199439)  EN   84
        R: AT BE CH DE FR GB IT LI LU NL SE
     DE 68918867     E  19941117 (199445)
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     EP 434713        A4 19920520 (199522)
     US 5792459       A  19980811 (199839)
     US 5814458       A  19980929 (199846)
     US 5817792       A  19981006 (199847)
ADT  EP 434713 A EP 1989-909916 19890821; EP 434713 B1 EP 1989-909916 19890821,
     WO 1989-US3605 19890821; DE 68918867 E DE 1989-618867 19890821, EP
     1989-909916 19890821, WO 1989-US3605 19890821; EP 434713 A4 EP 1989-909916
     ; US 5792459 A Cont of US 1988-243944 19880913, Cont of US 1993-6252
     19930119, Div ex US 1995-371618 19950112, US 1995-441184 19950515; US
     5814458 A Cont of US 1988-243944 19880913, Cont of US 1993-6252 19930119,
     US 1995-371618 19950112; US 5817792 A Cont of US 1988-243944 19880913,
     Cont of US 1993-6252 19930119, Div ex US 1995-371618 19950112, US
     1995-441356 19950515
FDT  EP 434713 B1 Based on WO 9002568; DE 68918867 E Based on EP 434713, Based
     on WO 9002568
PRAI US 1988-243944        19880913; US 1993-6252         19930119;
     US 1995-371618        19950112; US 1995-441184       19950515;
     US 1995-441356        19950515
AB   WO   9002568 A UPAB: 19930928
     The following are claimed: (A) an improved polypeptide analogue of human
     immunodeficiency virus type 1 (HIV-1) gp120 env or gp160 env, the
     improvement comprising the deletion of at least one epitope in a
     hypervariable domain, while retaining the domains conserved among HIV-1
     isolates; (B) a polypeptide comprising epitopes bound by antibodies to
     HIV-1 gp120 env or gp160 env and an amino acid sequence of formula
     C1-V1-V2-C2-V3 -C3-V4-C4-V5-C5 (I) (C1 = an amino acid sequence homologous
     to Ser29-Cys130 of HIV-1 SF2; C2 = an amino acid sequence homologous to
     Cys199-Leu291 of HIV-1 SF2; C3 = an amino acid sequence homologous to
     Ser366-Cys387 of HIV-1 SF2; C4 = an amino acid sequence homologous to
     Cys415-Gly456 of HIV-1 SF2; C5 = an amino acid sequence homologous to
     Phe466-Arg509 or Leu855 of HIV-1 SF2; V1, V4 = amino acid sequences of
     0-30 residues; V2 = an amino acid sequence of 0-50 residues; V3 = an amino
     acid sequence of 0-90 residues; V5 = an amino acid sequence of 0-10
     residues; with 9 specified proviso); (C) a DNA sequence encoding a
     polypeptide as in (A); (D) a cellular host comprising the DNA sequence of
     (C) under the control of transcriptional and translational control
     sequences.
          USE/ADVANTAGE - The polypeptides can be used to raise a high titre of
     antibodies which are reactive to all or most HIV-1 isolates.
     0/9

L2   ANSWER 12 OF 12  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   1987-179666 [26]   WPIDS
CR   1996-179247 [18]; 1997-414546 [38]
DNC  C1987-074773
TI   New polypeptide(s) with tissue plasminogen activator activity - also DNA
     sequences encoding the polypeptide(s), expression constructs and plasmids.
DC   B04 D16
IN   AFTING, E G; HAIGWOOD, N L; MULLENBACH, G; PAQUES, E P; AFTING, E;
     PAQUES, E
PA   (CHIR) CHIRON CORP; (BEHW) BEHRINGWERKE AG
CYC  18
PI   EP 227462        A  19870701 (198726)* EN   31
         R: AT BE CH DE ES FR GB GR IT LI LU NL SE
     AU 8666830       A  19870625 (198732)
     ZA 8609626       A  19870831 (198747)
     JP 62253380      A  19871105 (198750)
     DK 8606231       A  19870826 (198804)
     EP 227462        B  19920401 (199214)       38
         R: AT BE CH DE ES FR GB GR IT LI LU NL SE
     DE 3684680       G  19920507 (199220)
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     ES 2031826      T3 19930101 (199305)
     JP 07075583     A  19950320 (199520)      14
     JP 07087973     A  19950404 (199522)      13
     US 5439679      A  19950808 (199537)       8
     JP 2575369      B2 19970122 (199708)      12
     JP 09187287     A  19970722 (199739)      11
     JP 2680999      B2 19971119 (199751)      12
     JP 2681000      B2 19971119 (199751)      13
     JP 2769314      B2 19980625 (199830)      11
ADT  EP 227462 A EP 1986-310009 19861222; ZA 8609626 A ZA 1986-9626 19861222;
     JP 62253380 A JP 1986-316095 19861223; EP 227462 B EP 1986-310009
     19861222; ES 2031826 T3 EP 1986-310009 19861222; JP 07075583 A Div ex JP
     1986-316095 19861223, JP 1994-186171 19861223; JP 07087973 A Div ex JP
     1986-316095 19861223, JP 1994-186170 19861223; US 5439679 A Div ex US
     1985-812879 19851223, US 1994-208202 19940310; JP 2575369 B2 JP
     1986-316095 19861223; JP 09187287 A Div ex JP 1994-186171 19861223, JP
     1996-337365 19861223; JP 2680999 B2 Div ex JP 1986-316095 19861223, JP
     1994-186170 19861223; JP 2681000 B2 Div ex JP 1986-316095 19861223, JP
     1994-186171 19861223; JP 2769314 B2 Div ex JP 1994-186171 19861223, JP
     1996-337365 19861223
FDT  ES 2031826 T3 Based on EP 227462; JP 2575369 B2 Previous Publ. JP
     62253380; JP 2680999 B2 Previous Publ. JP 07087973; JP 2681000 B2 Previous
     Publ. JP 07075583; JP 2769314 B2 Previous Publ. JP 09187287
PRAI US 1985-812879      19851223; US 1994-208202       19940310
AB   EP    227462 A UPAB: 20030906
     (1) Polypeptide (I) is claimed having tissue plasminogen activator (tPA)
     activity in dissolving clots, having a specific activity at least 0.3 of
     that of natural tPA and at least one improved property as to specific
     activity, fibrin dependence of activity or inhibitor susceptibility, as a
     result of reducing the no. of glycosylation sites; deletion of at least 3
     amino acids at the C-terminus; or substitution of an amino acid at the
     cleavage site, in a naturally occurring polypeptide having tPA activity,
     pref. human tPA.
          (2) DNA sequence (II) encoding (I) is also new.  (3) An expression
     construct comprises in order of transcription, a transcriptional and
     translational initiation region; a DNA sequence (II) under transcriptional
     and translational regulatory control of the initiation region; and a
     termination region. Also claimed are: a plasmid having a replication
     system functional in a host and the expression construct; a viable cell
     contg. the plasmid; and a method for producing the DNA sequence.
          USE/ADVANTAGE - (I) are useful in the lysis of fibrin clots in
     treatment of thrombosis and prevention of clot formation by activation of
     plasminogen during operations etc. where the host may be susceptible to
     clot formation.  Reducing the amt. of glycosylation, esp. at the kringle
     sites, can enhance specific activity; C-terminus truncation enhances
     fibrin dependence and thus specific activity, so that the amt. of
     plasminogen cleaved to plasmin away from a clot is reduced and
     side-effects of plasmin avoided.  The susceptibility to tPA inhibitor may
     be reduced, e.g. by 25-90% c.f. natural tPA.  Lower amts. of (I) may be
     used c.f. natural tPA.  Use in diagnostics is also claimed.
     Dwg.0/0


=> file medline
COST IN U.S. DOLLARS                              SINCE FILE       TOTAL
                                                       ENTRY     SESSION
FULL ESTIMATED COST                                    59.64      102.15

FILE 'MEDLINE' ENTERED AT 20:54:28 ON 26 SEP 2006

 FILE LAST UPDATED: 26 Sep 2006 (20060926/UP).  FILE COVERS 1950 TO DATE.
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On December 11, 2005, the 2006 MeSH terms were loaded.

The MEDLINE reload for 2006 is now (26 Feb.) available.  For details
on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
See also:

   http://www.nlm.nih.gov/mesh/
   http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
   http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
   http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> e haigwood n l/au
E1           2      HAIGWOOD J T/AU
E2          10      HAIGWOOD N/AU
E3          40 --> HAIGWOOD N L/AU
E4           5      HAIGWOOD NANCY/AU
E5          13      HAIGWOOD NANCY L/AU
E6           1      HAIHARA YUKO/AU
E7           1      HAIHUA LIN/AU
E8           1      HAIJ M E/AU
E9           1      HAIJAMAE H/AU
E10          1      HAIJAZI H/AU
E11          1      HAIJE W/AU
E12         23      HAIJE W G/AU

=> s e3-e5
            40 "HAIGWOOD N L"/AU
             5 "HAIGWOOD NANCY"/AU
            13 "HAIGWOOD NANCY L"/AU
L3          58 ("HAIGWOOD N L"/AU OR "HAIGWOOD NANCY"/AU OR "HAIGWOOD NANCY
               L"/AU)

=> s l3 and (HIV or human immunodeficiency virus or SIV or simian immunodeficiency virus)
        163138 HIV
       1419595 HUMAN
        124744 IMMUNODEFICIENCY
        419259 VIRUS
         49374 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
          3248 SIV
         20943 SIMIAN
        124744 IMMUNODEFICIENCY
        419259 VIRUS
          4202 SIMIAN IMMUNODEFICIENCY VIRUS
                 (SIMIAN(W)IMMUNODEFICIENCY(W)VIRUS)
L4          50 L3 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
               IMMUNODEFICIENCY VIRUS)

=> s l4 and (env? or gp160 or gp120 or gp41)
        434927 ENV?
          1535 GP160
          6642 GP120
          2627 GP41
L5          35 L4 AND (ENV? OR GP160 OR GP120 OR GP41)
```

=> d l5,cbib,ab,1-35

L5 ANSWER 1 OF 35 MEDLINE on STN
2006526099. PubMed ID: 16951366. Evaluation of Passively Transferred, Nonneutralizing Antibody-Dependent Cellular Cytotoxicity-Mediating IgG in Protection of Neonatal Rhesus Macaques against Oral SIVmac251 Challenge. Florese Ruth H; Van Rompay Koen K A; Aldrich Kris; Forthal Donald N; Landucci Gary; Mahalanabis Madhumita; **Haigwood Nancy**; Venzon David; Kalyanaraman Vaniambadi S; Marthas Marta L; Robert-Guroff Marjorie. (Vaccine Branch and.) Journal of immunology (Baltimore, Md. : 1950), (2006 Sep 15) Vol. 177, No. 6, pp. 4028-36. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Previously, Ab-dependent cellular cytotoxicity (ADCC) was significantly correlated with reduced acute viremia upon intrarectal **SIV**(mac251) challenge of immunized rhesus macaques. To directly assess ADCC protective efficacy, six neonatal macaques were infused s.c. with immune IgG (220 mg/kg) purified from the immunized animals and positive for ADCC and Ab-dependent cell-mediated viral inhibition (ADCVI) activities. Six neonates received control IgG. The neonates were challenged twice orally with 10(5) 50% inhibiting tissue culture-infective dose of **SIV**(mac251) 2 days post-IgG infusion. At challenge, plasma of neonates that received immune IgG did not neutralize **SIV**(mac251) but had geometric mean ADCC titers of 48,130 and 232,850 against **SIV**(mac251)-infected and **gp120**-coated targets, respectively. Peak ADCVI activity varied from 62 to 81%. ADCC activity declined with the 2-wk IgG half-life but was boosted at wk 4, together with de novo ADCC-mediating Abs in controls, by postchallenge viremia. ADCVI activity was similarly induced. No protection, assessed by viral burdens, CD4 counts, and time to euthanasia was observed(.) Possible factors contributing to the discrepancy between the previous correlation and lack of protection here include: the high oral challenge dose compared with the 400-fold lower intrarectal dose; the challenge route with regard to viral dissemination and distribution of infused IgG; insufficient NK effector activity and/or poor functionality in newborns; insufficient immune IgG; and the possibility that the previous correlation of ADCC with protection was augmented by cellular immune responses also present at challenge. Future studies should explore additional challenge routes in juvenile macaques using higher amounts of potent IgG preparations.

L5 ANSWER 2 OF 35 MEDLINE on STN
2005690562. PubMed ID: 16379001. Consistent patterns of change during the divergence of **human immunodeficiency virus** type 1 **envelope** from that of the inoculated virus in simian/**human immunodeficiency virus**-infected macaques. Blay W M; Gnanakaran S; Foley B; Doria-Rose N A; Korber B T; **Haigwood N L**. (Department of Pathobiology, University of Washington, Seattle, WA 98195, USA.) Journal of virology, (2006 Jan) Vol. 80, No. 2, pp. 999-1014. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We have analyzed changes to proviral **Env gp120** sequences and the development of neutralizing antibodies (NAbs) during 1 year of simian/**human immunodeficiency virus** SHIV-89.6P infection in 11 Macaca nemestrina macaques. Seven macaques had significant **env** divergence from that of the inoculum, and macaques with greater divergence had higher titers of homologous NAbs. Substitutions in sequons encoding potential N-linked glycosylation sites (PNGs) were among the first to be established, although overall the total number of sequons did not increase significantly. The majority (19 of 23) of PNGs present in the inoculum were conserved in the sequences from all macaques. Statistically significant variations in PNGs occurred in multiple macaques within constrained regions we term "hot spots," resulting in the selection of sequences more similar to the B consensus. These included additions on

V1, the N-terminal side of V4, and the outer region of C2. Complex mutational patterns resulted in convergent PNG shifts in V2 and V5. Charge changes in **Env** V1V2, resulting in a net acidic charge, and a proline addition in V5 occurred in several macaques. Molecular modeling of the 89.6P sequence showed that the conserved glycans lie on the silent face of **Env** and that many are proximal to disulfide bonds, while PNG additions and shifts are proximal to the CD4 binding site. Nonsynonymous-to-synonymous substitution ratios suggest that these changes result from selective pressure. This longitudinal and cross-sectional study of mutations in **human immunodeficiency virus (HIV) env** in the SHIV background provides evidence that there are more constraints on the configuration of the glycan shield than were previously appreciated.

L5 ANSWER 3 OF 35 MEDLINE on STN
2005678218. PubMed ID: 16280707. High maternal **HIV**-1 viral load during pregnancy is associated with reduced placental transfer of measles IgG antibody. Farquhar Carey; Nduati Ruth; **Haigwood Nancy**; Sutton William; Mbori-Ngacha Dorothy; Richardson Barbra; John-Stewart Grace. (Department of Medicine, University of Washington, Seattle, WA 98104-2499, USA.. cfarg@u.washington.edu) . Journal of acquired immune deficiency syndromes (1999), (2005 Dec 1) Vol. 40, No. 4, pp. 494-7. Journal code: 100892005. ISSN: 1525-4135. Pub. country: United States. Language: English.

AB BACKGROUND: Studies among **HIV**-1-infected women have demonstrated reduced placental transfer of IgG antibodies against measles and other pathogens. As a result, infants born to women with **HIV**-1 infection may not acquire adequate passive immunity in utero and this could contribute to high infant morbidity and mortality in this vulnerable population. METHODS: To determine factors associated with decreased placental transfer of measles IgG, 55 **HIV**-1-infected pregnant women who were enrolled in a Nairobi perinatal **HIV**-1 transmission study were followed. Maternal CD4 count, **HIV**-1 viral load, and **HIV**-1-specific **gp41** antibody concentrations were measured antenatally and at delivery. Measles IgG concentrations were assayed in maternal blood and infant cord blood obtained during delivery to calculate placental antibody transfer. RESULTS: Among 40 women (73%) with positive measles titers, 30 (75%) were found to have abnormally low levels of maternofetal IgG transfer (<95%). High maternal **HIV**-1 viral load at 32 weeks' gestation and at delivery was associated with reductions in placental transfer ($P < 0.0001$ and $P = 0.0056$, respectively) and infant measles IgG concentrations in cord blood ($P < 0.0001$ and $P = 0.0073$, respectively). High maternal **HIV**-1-specific **gp41** antibody titer was also highly correlated with both decreased placental transfer ($P = 0.0080$) and decreased infant IgG ($P < 0.0001$). CONCLUSIONS: This is the first study to evaluate the relationship between maternal **HIV**-1 viremia, maternal **HIV**-1 antibody concentrations, and passive immunity among **HIV**-1-exposed infants. These data support the hypothesis that high **HIV**-1 viral load during the last trimester may impair maternofetal transfer of IgG and increases risk of measles and other serious infections among **HIV**-1-exposed infants.

L5 ANSWER 4 OF 35 MEDLINE on STN
2005576191. PubMed ID: 16254780. AIDS Vaccine 2005. **Haigwood Nancy L**; Doria-Rose Nicole. (Seattle Biomedical Research Institute, Seattle, WA 98109, USA.. nancy.haigwood@sbri.org) . IDrugs : the investigational drugs journal, (2005 Nov) Vol. 8, No. 11, pp. 898-900. Journal code: 100883655. ISSN: 1369-7056. Pub. country: England: United Kingdom. Language: English.

L5 ANSWER 5 OF 35 MEDLINE on STN
2005462342. PubMed ID: 16128920. Infection with a molecularly cloned SIVsm virus elicits high titer homologous neutralizing antibodies with heterologous neutralizing activity. Mahalanabis M; Hirsch V M; **Haigwood N L**. (Seattle Biomedical Research Institute, Seattle, WA 98109-5219, USA.) Journal of medical primatology, (2005 Oct) Vol. 34, No. 5-6, pp. 253-61.

Journal code: 0320626. ISSN: 0047-2565. Pub. country: Denmark. Language: English.

AB We have evaluated the homologous and heterologous neutralizing antibody response in a cohort of six Macaca nemestrina infected with the cloned virus SIVsm62d that showed different levels of **envelope** diversification. Two progressor macaques developed AIDS by 1.5 years post-inoculation and four non-progressors were asymptomatic for 3 years of follow-up. All macaques developed high titers of neutralizing antibodies against homologous SIVsm viruses and intermediate titers against SIVsmB670. Heterologous virus neutralization of SIVmac, SIVmne, and **HIV**-2 was detected at much lower levels in both progressor macaques; only one of four non-progressors had evidence for broader neutralizing antibody activity. We noted changes in potential N-linked glycosylation (PNG) sites in V1/V2, C2, and V4 that were common to multiple macaques. These results support a model for viral neutralization where heterologous neutralization is, in part, driven by a strong homologous response and may be coupled to changes in PNG sites in **envelope**.

L5 ANSWER 6 OF 35 MEDLINE on STN

2005436504. PubMed ID: 16103173. **Human immunodeficiency virus** type 1 subtype B ancestral **envelope** protein is functional and elicits neutralizing antibodies in rabbits similar to those elicited by a circulating subtype B **envelope**. Doria-Rose N A; Learn G H; Rodrigo A G; Nickle D C; Li F; Mahalanabis M; Hensel M T; McLaughlin S; Edmonson P F; Montefiori D; Barnett S W; **Haigwood N L**; Mullins J I. (Seattle Biomedical Research Institute, Washington, USA.) Journal of virology, (2005 Sep) Vol. 79, No. 17, pp. 11214-24. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) is a difficult target for vaccine development, in part because of its ever-expanding genetic diversity and attendant capacity to escape immunologic recognition. Vaccine efficacy might be improved by maximizing immunogen antigenic similarity to viruses likely to be encountered by vaccinees. To this end, we designed a prototype **HIV**-1 **envelope** vaccine using a deduced ancestral state for the **env** gene. The ancestral state reconstruction method was shown to be >95% accurate by computer simulation and 99.8% accurate when estimating the known inoculum used in an experimental infection study in rhesus macaques. Furthermore, the deduced ancestor gene differed from the set of sequences used to derive the ancestor by an average of 12.3%, while these latter sequences were an average of 17.3% different from each other. A full-length ancestral subtype B **HIV**-1 **env** gene was constructed and shown to produce a glycoprotein of 160 kDa that bound and fused with cells expressing the **HIV**-1 coreceptor CCR5. This **Env** was also functional in a virus pseudotype assay. When either **gp160**- or gp140-expressing plasmids and recombinant **gp120** were used to immunize rabbits in a DNA prime-protein boost regimen, the artificial gene induced immunoglobulin G antibodies capable of weakly neutralizing heterologous primary **HIV**-1 strains. The results were similar for rabbits immunized in parallel with a natural isolate, **HIV**-1 SF162. Further design efforts to better present conserved neutralization determinants are warranted.

L5 ANSWER 7 OF 35 MEDLINE on STN

2004547799. PubMed ID: 15175256. Tracking global patterns of N-linked glycosylation site variation in highly variable viral glycoproteins: **HIV**, **SIV**, and HCV **envelopes** and influenza hemagglutinin. Zhang Ming; Gaschen Brian; Blay Wendy; Foley Brian; **Haigwood Nancy**; Kuiken Carla; Korber Bette. (Theoretical Biology Group, Los Alamos National Laboratory, Los Alamos, NM 87544, USA.) Glycobiology, (2004 Dec) Vol. 14, No. 12, pp. 1229-46. Electronic Publication: 2004-06-02. Journal code: 9104124. ISSN: 0959-6658. Pub. country: England: United Kingdom. Language: English.

AB Human and simian immunodeficiency viruses (**HIV** and **SIV**), influenza

virus, and hepatitis C virus (HCV) have heavily glycosylated, highly variable surface proteins. Here we explore N-linked glycosylation site (sequon) variation at the population level in these viruses, using a new Web-based program developed to facilitate the sequon tracking and to define patterns (www.**hiv**.lanl.gov). This tool allowed rapid visualization of the two distinctive patterns of sequon variation found in **HIV**-1, **HIV**-2, and **SIV** CPZ. The first pattern (fixed) describes readily aligned sites that are either simply present or absent. These sites tend to be occupied by high-mannose glycans. The second pattern (shifting) refers to sites embedded in regions of extreme local length variation and is characterized by shifts in terms of the relative position and local density of sequons; these sites tend to be populated by complex carbohydrates. **HIV**, with its extreme variation in number and precise location of sequons, does not have a net increase in the number of sites over time at the population level. Primate lentiviral lineages have host species-dependent levels of sequon shifting, with **HIV**-1 in humans the most extreme. HCV E1 and E2 proteins, despite evolving extremely rapidly through point mutation, show limited sequon variation, although two shifting sites were identified. Human influenza A hemagglutinin H3 HA1 is accumulating sequons over time, but this trend is not evident in any other avian or human influenza A serotypes.

L5 ANSWER 8 OF 35 MEDLINE on STN
2004258528. PubMed ID: 15157361. Protective immunity to **SIV** challenge elicited by vaccination of macaques with multigenic DNA vaccines producing virus-like particles. Mossman Sally P; Pierce Christopher C; Watson Andrew J; Robertson Michael N; Montefiori David C; Kuller Larene; Richardson Barbra A; Bradshaw Jeffrey D; Munn Robert J; Hu Shiu-Lok; Greenberg Philip D; Benveniste Raoul E; **Haigwood Nancy L**. (Seattle Biomedical Research Institute, Seattle, Washington 98109, USA.) AIDS research and human retroviruses, (2004 Apr) Vol. 20, No. 4, pp. 425-34. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB We utilized **SIV**(mne) infection of Macaca fascicularis to assess the efficacy of DNA vaccination alone, and as a priming agent in combination with subunit protein boosts. All **SIV**(mne) structural and regulatory genes were expressed using the human cytomegalovirus Immediate Early-1 promoter in plasmids that directed the formation of virus-like particles in vitro. Macaques (n = 4) were immunized intradermally and intramuscularly four times over 36 weeks with 3 mg plasmid DNA. A second group (n = 4) received two DNA priming inoculations followed by two intramuscular boosts consisting of 250 microg recombinant **Env gp160** and 250 microg recombinant Gag-Pol particles in MF-59 adjuvant. These regimens elicited modest cellular immunity prior to challenge. Humoral immune responses to **Env gp160** were elicited and sustained by both vaccine protocols, and as expected antibody titers were higher in the protein subunit-boosted animals. Neutralizing antibodies prior to challenge were measurable in two of four subunit-boosted macaques. The two vaccine regimens elicited comparable helper T cell responses at the time of challenge. Vaccinees and mock-immunized controls (n = 4) were challenged intrarectally at week 38 with uncloned **SIV**(mne). Following challenge all macaques became infected, but both vaccine regimens resulted in reduced peak virus loads (p = 0.07) and significantly improved maintenance of peripheral CD4(+) T cell counts postchallenge (p = 0.007, DNA alone and p = 0.01, all vaccinees). There was no significant difference between the two vaccine groups in levels of plasma viremia or maintenance of CD4(+) T cell counts postchallenge.

L5 ANSWER 9 OF 35 MEDLINE on STN
2004242473. PubMed ID: 15140996. Passive immunotherapy in **simian immunodeficiency virus**-infected macaques accelerates the development of neutralizing antibodies. **Haigwood Nancy L**; Montefiori David C; Sutton William F; McClure Janela; Watson Andrew J; Voss Gerald; Hirsch Vanessa M;

Richardson Barbra A; Letvin Norman L; Hu Shiu-Lok; Johnson Philip R. (Seattle Biomedical Research Institute, 307 Westlake Ave. N., Suite 500, Seattle, WA 98109-5219, USA.. Nancy.Haigwood@sbri.org) . Journal of virology, (2004 Jun) Vol. 78, No. 11, pp. 5983-95. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Passively transferred neutralizing antibodies can block lentivirus infection, but their role in postexposure prophylaxis is poorly understood. In this nonhuman-primate study, the effects of short-term antibody therapy on 5-year disease progression, virus load, and host immunity were explored. We reported previously that postinfection passive treatment with polyclonal immune globulin with high neutralizing titers against SIVsmE660 (SIVIG) significantly improved the 67-week health of SIVsmE660-infected Macaca mulatta macaques. Four of six treated macaques maintained low or undetectable levels of virus in plasma, compared with one of ten controls, while two rapid progressors controlled viremia only as long as the SIVIG was present. SIVIG treatment delayed the de novo production of **envelope (Env)**-specific antibodies by 8 weeks (13). We show here that differences in disease progression were also significant at 5 years postinfection, excluding rapid progressors (P = 0.05). Macaques that maintained </=10(3) virus particles per ml of plasma and </=30 infectious virus particles per 10(6) mononuclear cells from peripheral blood and lymph nodes had delayed disease onset. All macaques that survived beyond 18 months had measurable Gag-specific CD8(+) cytotoxic T cells, regardless of treatment. Humoral immunity in survivors beyond 20 weeks was strikingly different in the SIVIG and control groups. Despite a delay in **Env**-specific binding antibodies, de novo production of neutralizing antibodies was significantly accelerated in SIVIG-treated macaques. Titers of de novo neutralizing antibodies at week 12 were comparable to levels achieved in controls only by week 32 or later. Acceleration of de novo **simian immunodeficiency virus** immunity in the presence of passively transferred neutralizing antibodies is a novel finding with implications for postexposure prophylaxis and vaccines.

L5 ANSWER 10 OF 35 MEDLINE on STN
2004184194. PubMed ID: 15080182. Role of neutralizing antibodies in **HIV** infection. **Haigwood Nancy L**; Stamatatos Leonidas. (Seattle Biomedical Research Institute, Seattle, WA 98103, USA.) AIDS (London, England), (2003) Vol. 17 Suppl 4, pp. S67-71. Ref: 36. Journal code: 8710219. ISSN: 0269-9370. Pub. country: England: United Kingdom. Language: English.

L5 ANSWER 11 OF 35 MEDLINE on STN
2003479429. PubMed ID: 14557642. Multigene DNA priming-boosting vaccines protect macaques from acute CD4+-T-cell depletion after simian-**human immunodeficiency virus** SHIV89.6P mucosal challenge. Doria-Rose N A; Ohlen C; Polacino P; Pierce C C; Hensel M T; Kuller L; Mulvania T; Anderson D; Greenberg P D; Hu S-L; **Haigwood N L**. (Seattle Biomedical Research Institute, University of Washington, Seattle, Washington 98109, USA.) Journal of virology, (2003 Nov) Vol. 77, No. 21, pp. 11563-77. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We evaluated four priming-boosting vaccine regimens for the highly pathogenic simian **human immunodeficiency virus** SHIV89.6P in Macaca nemestrina. Each regimen included gene gun delivery of a DNA vaccine expressing all SHIV89.6 genes plus **Env gp160** of SHIV89.6P. Additional components were two recombinant vaccinia viruses, expressing SHIV89.6 Gag-Pol or **Env gp160**, and inactivated SHIV89.6 virus. We compared (i) DNA priming/DNA boosting, (ii) DNA priming/inactivated virus boosting, (iii) DNA priming/vaccinia virus boosting, and (iv) vaccinia virus priming/DNA boosting versus sham vaccines in groups of 6 macaques. Prechallenge antibody responses to **Env** and Gag were strongest in the groups that received vaccinia virus priming or boosting. Cellular immunity to SHIV89.6 peptides was measured by enzyme-linked immunospot

assay; strong responses to Gag and **Env** were found in 9 of 12 vaccinia virus vaccinees and 1 of 6 DNA-primed/inactivated-virus-boosted animals. Vaccinated macaques were challenged intrarectally with 50 50% animal infectious doses of SHIV89.6P 3 weeks after the last immunization. All animals became infected. Five of six DNA-vaccinated and 5 of 6 DNA-primed/particle-boosted animals, as well as all 6 controls, experienced severe CD4(+)-T-cell loss in the first 3 weeks after infection. In contrast, DNA priming/vaccinia virus boosting and vaccinia virus priming/DNA boosting vaccines both protected animals from disease: 11 of 12 macaques had no loss of CD4(+) T cells or moderate declines. Virus loads in plasma at the set point were significantly lower in vaccinia virus-primed/DNA-boosted animals versus controls (P = 0.03). We conclude that multigene vaccines delivered by a combination of vaccinia virus and gene gun-delivered DNA were effective against SHIV89.6P viral challenge in M. nemestrina.

L5 ANSWER 12 OF 35 MEDLINE on STN

2002109697. PubMed ID: 11836389. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-**human immunodeficiency virus** challenge following passive transfer of high-titered neutralizing antibodies. Nishimura Yoshiaki; Igarashi Tatsuhiko; **Haigwood Nancy**; Sadjadpour Reza; Plishka Ron J; Buckler-White Alicia; Shibata Riri; Martin Malcolm A. (Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 9000 Rockville Pike, Bldg. 4 Rm. 315, Bethesda, MD 20892, USA.) Journal of virology, (2002 Mar) Vol. 76, No. 5, pp. 2123-30. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We previously reported that high-titered neutralizing antibodies directed against the **human immunodeficiency virus** type 1 (**HIV**-1) **envelope** can block the establishment of a **simian immunodeficiency virus** (**SIV**)/**HIV** chimeric virus (SHIV) infection in two monkeys following passive transfer (R. Shibata et al., Nat. Med. 5:204-210, 1999). In the present study, increasing amounts of neutralizing immunoglobulin G (IgG) were administered to 15 pig-tailed macaques in order to obtain a statistically valid protective neutralization endpoint titer in plasma. Using an in vitro assay which measures complete neutralization of the challenge SHIV, we correlated the titers of neutralizing antibodies in plasma at the time of virus inoculation (which ranged from 1:3 to 1:123) with the establishment of infection in virus-challenged animals. Ten of 15 monkeys in the present experiment were virus free as a result of neutralizing IgG administration as monitored by DNA PCR (peripheral blood mononuclear cells and lymph node cells), RNA PCR (plasma), virus isolation, and the transfer of lymph node cell suspensions (10(8) cells) plus 8 ml of whole blood from protected animals to naive macaques. The titer of neutralizing antibodies in the plasma calculated to protect 99% of virus-challenged monkeys was 1:38.

L5 ANSWER 13 OF 35 MEDLINE on STN

2000059060. PubMed ID: 10593487. Immunization against SIVmne in macaques using multigenic DNA vaccines. Mossman S P; Pierce C C; Robertson M N; Watson A J; Montefiori D C; Rabin M; Kuller L; Thompson J; Lynch J B; Morton W R; Benveniste R E; Munn R; Hu S L; Greenberg P; **Haigwood N L**. (Seattle Biomedical Research Institute, WA 98109-1651, USA.) Journal of medical primatology, (1999 Aug-Oct) Vol. 28, No. 4-5, pp. 206-13. Journal code: 0320626. ISSN: 0047-2565. Pub. country: Denmark. Language: English.

AB All structural and regulatory genes of SIVmne were cloned into mammalian expression vectors to optimize expression in vitro and immunogenicity in mice. Macaca fascicularis were immunized four times with plasmid DNA (n = 4), or two DNA priming inoculations followed by two boosts of recombinant **gp160** plus Gag-Pol particles (n = 4). Following intrarectal challenge with SIVmne, all macaques became infected. Three monkeys immunized with

DNA alone maintained low plasma virus loads by 1 year post-challenge; the fourth exhibited high virus loads and significant CD4+ cell decline. Two of the DNA plus boost and three control macaques had high virus loads and associated CD4+ cell decline. Both vaccine protocols elicited antibodies and comparable helper T-cell proliferative responses to **gp160**. Cytokine mRNA levels in activated peripheral blood mononuclear cells (PBMC) taken at time of challenge suggested a dominant T helper (Th) 1 state in three DNA-immunized and one protein-boosted macaque, which correlated with low virus loads and high CD4+ cell counts post-challenge.

L5 ANSWER 14 OF 35 MEDLINE on STN
1999217703. PubMed ID: 10203053. Protection from pathogenic **SIV** challenge using multigenic DNA vaccines. **Haigwood N L**; Pierce C C; Robertson M N; Watson A J; Montefiori D C; Rabin M; Lynch J B; Kuller L; Thompson J; Morton W R; Benveniste R E; Hu S L; Greenberg P; Mossman S P. (Seattle Biomedical Research Institute, WA 98109, USA.. haigwood@u.washington.edu) . Immunology letters, (1999 Mar) Vol. 66, No. 1-3, pp. 183-8. Journal code: 7910006. ISSN: 0165-2478. Pub. country: Netherlands. Language: English.

AB To assess DNA immunization as a strategy for protecting against **HIV** infection in humans, we utilized SIVmne infection of Macaca fascicularis as a vaccine challenge model with moderate pathogenic potential. We compared the efficacy of DNA immunization alone and in combination with subunit protein boosts. All of the structural and regulatory genes of SIVmne clone 8 were cloned into mammalian expression vectors under the control of the CMV IE-1 promoter. Eight M. fascicularis were immunized twice with 3 mg of plasmid DNA divided between two sites; intramuscular and intradermal. Four primed macaques received a further two DNA immunizations at weeks 16-36, while the second group of four were boosted with 250 microg recombinant **gp160** plus 250 microg recombinant Gag-Pol particles formulated in MF-59 adjuvant. Half of the controls received four immunizations of vector DNA; half received two vector DNA and two adjuvant immunizations. As expected, humoral immune responses were stronger in the macaques receiving subunit boosts, but responses were sustained in both groups. Significant neutralizing antibody titers to SIVmne were detected in one of the subunit-boosted animals and in none of the DNA-only animals prior to challenge. T-cell proliferative responses to **gp160** and to Gag were detected in all immunized animals after three immunizations, and these responses increased after four immunizations. Cytokine profiles in PHA-stimulated PBMC taken on the day of challenge showed trends toward Thl responses in 2/4 macaques in the DNA vaccinated group and in 1/4 of the DNA plus subunit vaccinated macaques; Th2 responses in 3/4 DNA plus subunit-immunized macaques; and Th0 responses in 4/4 controls. In bulk CTL culture, **SIV** specific lysis was low or undetectable, even after four immunizations. However, stable **SIV** Gag-Pol- and **env**-specific T-cell clones (CD3+ CD8+) were isolated after only two DNA immunizations, and Gag-Pol- and Nef-specific CTL lines were isolated on the day of challenge. All animals were challenged at week 38 with SIVmne uncloned stock by the intrarectal route. Based on antibody anamnestic responses (western, ELISA, and neutralizing antibodies) and virus detection methods (co-culture of PBMC and LNMC, nested set PCR- of DNA from PBMC and LNMC, and plasma QC-PCR), there were major differences between the groups in the challenge outcome. Surprisingly, sustained low virus loads were observed only in the DNA group, suggesting that four immunizations with DNA only elicited more effective immune responses than two DNA primes combined with two protein boosts. Multigenic DNA vaccines such as these, bearing all structural and regulatory genes, show significant promise and may be a safe alternative to live-attenuated vaccines.

L5 ANSWER 15 OF 35 MEDLINE on STN
1998296649. PubMed ID: 9632993. Humoral immunity to **HIV**, **SIV**, and

SHIV. **Haigwood N L**; Zolla-Pazner S. (Seattle Biomedical Research Institute, WA 98121, USA.) AIDS (London, England), (1998) Vol. 12 Suppl A, pp. S121-32. Ref: 137. Journal code: 8710219. ISSN: 0269-9370. Pub. country: ENGLAND: United Kingdom. Language: English.

L5 ANSWER 16 OF 35 MEDLINE on STN
96407300. PubMed ID: 8811353. Passive immune globulin therapy in the **SIV**/macaque model: early intervention can alter disease profile. **Haigwood N L**; Watson A; Sutton W F; McClure J; Lewis A; Ranchalis J; Travis B; Voss G; Letvin N L; Hu S L; Hirsch V M; Johnson P R. (Department of Immunodeficiency and Immunosuppression, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, Washington 98121, USA. Nancy L.. Haigwood@ccmail.bms.com) . Immunology letters, (1996 Jun) Vol. 51, No. 1-2, pp. 107-14. Journal code: 7910006. ISSN: 0165-2478. Pub. country: Netherlands. Language: English.

AB One of the major questions in AIDS is the role that the host immune system and the virus play in the dynamics of infection and the development of AIDS in an infected individual. In order to test the role of antibody in controlling viral infection, high-dose **SIV**-immune globulin was passively transferred to infected macaques early in infection. Immune globulin purified from the plasma of an **SIV**-infected long-term non-progressor macaque (SIVIG) or a pool of normal immune globulin (normal Ig) was infused into SIVsmE660-infected macaques (170 mg/kg) at one and fourteen days post infection. Animals were monitored for **SIV**-specific antibodies, viremia, plasma antigenemia, and clinical course. All animals were infected by **SIV**. At 16 months post infection, five macaques in the combined control groups have been euthanized, one as a rapid progressor with debilitating disease at 20 weeks post infection. Four macaques from the comparison groups have signs of AIDS, accompanied by high and increasing levels of virus and p27 antigenemia. One of the ten control animals had a very low virus load in plasma and peripheral blood and lymph node mononuclear cells at all times tested and has remained disease-free. In the SIVIG treatment group, two macaques were euthanized at 18-20 weeks due to AIDS, rapid progressors to disease. Three macaques in the SIVIG group had an initial high level of virus in plasma, peripheral blood mononuclear cells (PBMC), and lymph node mononuclear cells (LNMC), which dropped to baseline at 6 weeks post infection and has remained very low or negative for 16 months, a disease profile which has not been observed in untreated animals in this model to date. These macaques have remained clinically healthy. The sixth treated animal is also healthy, with very low virus burden that is detectable only by nested set polymerase chain reaction (PCR). All SIVIG-treated macaques had no detectable p27 plasma antigenemia for the first 10 weeks of infection, demonstrating that the IgG effectively complexed with the virus. The immunological correlates in the treated animals include development of de novo virus-specific antibodies and/or cytotoxic T cell (CTL), both of which are hallmarks of long term non-progressors. The two SIVIG-treated macaques that progress to disease rapidly had no detectable de novo humoral immune responses, as is often seen in rapid **HIV** disease in humans. **Envelope**-specific and virus neutralizing antibodies alone were not sufficient to prevent disease progression, as the plasma of both non-progressors as well as progressors had high titers of **envelope**-specific and neutralizing antibodies against SIVsmE660. Poor clinical prognosis was associated with moderate to high and increasing virus loads in plasma, PBMC, and lymph nodes. Good clinical prognosis correlated with low or undetectable post acute viremia in the peripheral blood and lymph nodes. We hypothesize that SIVIG reduced the spread of virus by eliminating or reducing plasma virus through immune complexes during the first four to 8 weeks of infection and then maintaining this low level of viremia until the host immune response was capable of virus control. Reduction of virus burden early in infection by passive IgG can alter disease outcome in **SIV** infection of macaques. Modifications of this strategy may lead to effective early

treatment of **HIV**-1 infection in humans.

L5 ANSWER 17 OF 35 MEDLINE on STN
96188305. PubMed ID: 8605050. Resistance of chimpanzees immunized with recombinant gp120SF2 to challenge by **HIV**-1SF2. el-Amad Z; Murthy K K; Higgins K; Cobb E K; **Haigwood N L**; Levy J A; Steimer K S. (Chiron Biocine, Emeryville, California 94608-2916, USA.) AIDS (London, England), (1995 Dec) Vol. 9, No. 12, pp. 1313-22. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

AB OBJECTIVE: To determine whether vaccination with recombinant **HIV**-1SF2 **gp120** in a novel oil-in-water adjuvant emulsion, MF59, protects chimpanzees against challenge with **HIV**-1SF2, the homologous virus isolate. METHODS: Two vaccinated chimpanzees and two control animals were challenged with 25-50 animal infectious doses of a stock of **HIV**-1SF2 that had been grown in mitogen-activated human peripheral blood mononuclear cells (PBMC). The animals were monitored by a series of serologic [enzyme-linked immunosorbent assay (ELISA), Western blot, and neutralization assays] and virologic [virus culture, RNA and DNA polymerase chain reaction (PCR)] assays for infection. RESULTS: Both control animals showed evidence of seroconversion in ELISA and Western blot assays. In addition, virus was detected in the early, acute phase of infection of both control animals by (1) plasma RNA PCR, (2) virus culture, and (3) PBMC DNA PCR assays. One vaccinated animal showed no serologic or virologic evidence of infection. The other vaccinated animal has not seroconverted, and there was no evidence of plasma viremia. However, virus was detected at early timepoints in this animal's PBMC, and transient lymphoproliferation to **HIV**-1 proteins not in the vaccine was observed. These observations suggest that the former animal was protected from challenge while the latter may have experienced a transient or curtailed infection. CONCLUSION: Two types of vaccine-induced protective immune responses were observed when chimpanzees immunized with rgp120SF2 were challenged with the homologous virus isolate: a response consistent with the 'sterilizing immune response' documented in the chimpanzee model in previous studies, as well as one that did not completely protect from infection, showing curtailment of the acute phase and a failure of the animal to seroconvert.

L5 ANSWER 18 OF 35 MEDLINE on STN
95151367. PubMed ID: 7848685. Fine analysis of humoral antibody response to **envelope** glycoprotein of **SIV** in infected and vaccinated macaques. Silvera P; Flanagan B; Kent K; Rud E; Powell C; Corcoran T; Bruck C; Thiriart C; **Haigwood N L**; Stott E J. (National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, United Kingdom.) AIDS research and human retroviruses, (1994 Oct) Vol. 10, No. 10, pp. 1295-304. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB To characterize the serological response to **SIV envelope**, induced by vaccination with different **envelope** immunogens or by **SIV** infection, plasma samples from 11 cynomolgus macaques infected with **simian immunodeficiency virus** (**SIV**) and from 16 macaques vaccinated with three different recombinant **envelope** proteins were analyzed by (1) ELISA, using a variety of antigens including overlapping peptides encompassing the entire sequence of the **envelope** protein of **SIV**, and (2) competition assays, using neutralizing monoclonal antibodies to **SIV gp120**. Seven regions of **SIV envelope** were predicted to be antigenic. Peptides representing four of these, in the second and third variable regions (V2 and V3) and the fourth constant (C4) region of **gp120** and the Gnann region of **gp41**, were recognized by the majority of sera from infected and vaccinated animals. Additional antigenic regions were identified in the first and fourth variable domains (V1 and V4) and the carboxy terminus (C5) of **gp120** and in three additional regions of **gp41**. Most infected and vaccinated animals made antibodies that

competed with the binding of the three conformational MAbs. Among the vaccinated groups, antibodies induced by vaccination with precursor glycoproteins (gp140 or **gp160**) recognized several additional **gp120** epitopes when compared with antibodies induced by external glycoprotein gp130. Sera from infected animals showed a more restricted **gp120** response (17 of 46 peptides recognized) compared to animals vaccinated with precursor glycoproteins (31 peptides recognized). The converse was true for antibodies to **gp41**. Sera from animals vaccinated with recombinant gp140, produced in insect cells, were the only group that failed to compete with the binding of conformational MAbs. Finally, the development of antibodies to specific epitopes of **gp120** and **gp41** revealed differences between long-term survivors and nonsurvivors, implying that responses to specific epitopes may be important in conferring resistance to disease progression.

L5 ANSWER 19 OF 35 MEDLINE on STN
94257325. PubMed ID: 8198872. Reduced virus load in rhesus macaques immunized with recombinant **gp160** and challenged with **simian immunodeficiency virus**. Ahmad S; Lohman B; Marthas M; Giavedoni L; el-Amad Z; **Haigwood N L**; Scandella C J; Gardner M B; Luciw P A; Yilma T. (Department of Pathology, Microbiology, and Immunology, University of California at Davis 95616.) AIDS research and human retroviruses, (1994 Feb) Vol. 10, No. 2, pp. 195-204. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB As a safe alternative to inactivated and live-attenuated whole-virus **SIV** vaccines, we have evaluated the potential of SIVmac239 **gp160** expressed by recombinant vaccinia virus (vSIVgp160) and baculovirus (bSIVgp160) to protectively immunize rhesus macaques against intravenous (i.v.) infection with pathogenic SIVmac isolates. Macaques were immunized with live vSIVgp160 and/or bSIVgp160 protein partially purified from insect cells. The challenge viruses, propagated in rhesus peripheral blood mononuclear cells, consisted of the molecular clone SIVmac239 and another genetically similar, uncloned isolate, SIVmac251. Although antibodies that bind gp130 were induced in all animals following immunization with SIVgp160, neutralizing antibodies were undetectable 1 week prior to virus challenge. These results differ from those for macaques vaccinated with inactivated, whole **SIV**. All animals became infected after i.v. inoculation with 1-10 AID50 of either challenge virus. For animals challenged with SIVmac251, but not those challenged with SIVmac239, the cell-free infectious virus load in plasma of vSIVgp160-primed, bSIVgp160-boosted macaques was significantly lower than in unimmunized controls at 2 weeks postchallenge. Virus virulence, immunization regimen, and challenge with homologous or heterologous virus are factors critical to the outcome of the study. Immunization with surface glycoprotein may not necessarily provide protective immunity against infection but may reduce virus load. The relationship between reduction in virus load by vaccination and delay in onset of disease remains to be determined.

L5 ANSWER 20 OF 35 MEDLINE on STN
94089664. PubMed ID: 7505441. V3 variability can influence the ability of an antibody to neutralize or enhance infection by diverse strains of **human immunodeficiency virus** type 1. Kliks S C; Shioda T; **Haigwood N L**; Levy J A. (Department of Medicine, University of California, School of Medicine, San Francisco 94143-0128.) Proceedings of the National Academy of Sciences of the United States of America, (1993 Dec 15) Vol. 90, No. 24, pp. 11518-22. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Human monoclonal antibodies (mAbs) to two contiguous epitopes in the V3 loop of the **human immunodeficiency virus** type 1 (**HIV-1**) **envelope** have shown different effects on three distinct strains of the virus: neutralization, enhancement, or resistance to both processes. Only one amino acid in the mAb epitopes proximal to the crown of the V3 loop was

different among these three strains. Substitution of this amino acid in the neutralizable strain with the amino acid of the neutralization-resistant strain or the enhanceable strain resulted in loss of both activities. The conversion of this single amino acid in the neutralization-resistant strain to that of the amino acid found in the neutralization-sensitive strain did not confer the ability for the virus to be neutralized. However, additional changes in neighboring amino acids in the V3 loop succeeded in conferring the neutralization capability. These observations indicate that one antibody species can exert three different effects on various **HIV**-1 strains. They could explain the emergence of neutralization "escape" variants in the presence of the neutralizing antibodies. Moreover, the results suggest caution in immunization of individuals with the **envelope** region from one strain since the antibodies induced may show a neutralizing effect against the homologous strain but enhancing effects against other unrelated strains.

L5 ANSWER 21 OF 35 MEDLINE on STN

93271839. PubMed ID: 1726963. Importance of conformation on the neutralizing antibody response to **HIV-1 gp120**. Steimer K S; **Haigwood N L**. (Chiron Corporation, Emeryville, California 94608-2916.) Biotechnology therapeutics, (1991) Vol. 2, No. 1-2, pp. 63-89. Journal code: 8918082. ISSN: 0898-2848. Pub. country: United States. Language: English.

AB We have investigated the role of conformation of **HIV**-1 **gp120** on its potential efficacy as a subunit vaccine. The questions that we set out to answer were: 1) Are there neutralizing antibodies directed to conformational epitopes in **gp120**? 2) If so, what is the spectrum of virus isolates neutralized by these antibodies? 3) Is a conformationally correct **gp120** subunit more effective in the induction of neutralizing antibodies than a denatured subunit? 4) Does native **gp120** subunit vaccination induce a broader neutralizing response than a **gp120** antigen that cannot display conformational epitopes? To address these questions, we characterized the **gp120**-specific antibody response of **HIV**-1-infected humans and of experimental animals immunized with recombinant native and nonnative **gp120** subunits. Two versions of recombinant **gp120** produced from the **HIV**-SF2 isolate of **HIV**-1 were employed in these studies: 1) a nonglycosylated, denatured version produced in genetically engineered yeast, which we presume is capable of presenting only linear determinants, and 2) a fully glycosylated, native version, produced in genetically engineered mammalian cells, that is capable of displaying linear as well as conformational epitopes. Antibodies directed exclusively to conformational epitopes in **gp120** were purified from pooled **HIV** antibody-positive human sera using these two versions of **HIV**-SF2 **gp120**. These antibodies exhibited neutralizing activity, and this activity was effective in the neutralization of a different, broader spectrum of **HIV**-1 isolates than that of antibodies to linear determinants in **gp120** purified from the same serum pool. When these two versions of **HIV**-SF2 **gp120** were used as subunit immunogens in baboons, clear differences in their abilities to elicit neutralizing antibodies were observed. The native version was more effective in the induction of neutralizing antibodies effective against **HIV**-SF2, the homologous virus isolate. The isolate specificity of the neutralizing response to these two versions of **HIV**-SF2 **gp120** also differed. The nonglycosylated version induced neutralizing antibodies that were effective against only the isolate, or closely related isolates, from which the antigen was derived. In contrast, the native version induced a neutralizing response that was effective against a broad panel of **HIV**-1 isolates, including at least one isolate that one would not expect to be neutralized by antibodies to the PND of **HIV**-SF2 **gp120**.

L5 ANSWER 22 OF 35 MEDLINE on STN

93103822. PubMed ID: 1281658. Immunization of primates with native,

recombinant **HIV**-SF2 **gp120** generates broadly effective neutralizing antibodies directed to conformational epitopes. Steimer K S; **Haigwood N L**. (Chiron Corporation, Emeryville, CA.) AIDS research and human retroviruses, (1992 Aug) Vol. 8, No. 8, pp. 1391. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

L5 ANSWER 23 OF 35 MEDLINE on STN
93100846. PubMed ID: 8416384. Immune response of rhesus macaques to recombinant **simian immunodeficiency virus** gp130 does not protect from challenge infection. Giavedoni L D; Planelles V; **Haigwood N L**; Ahmad S; Kluge J D; Marthas M L; Gardner M B; Luciw P A; Yilma T D. (Department of Veterinary Microbiology, University of California, Davis 95616.) Journal of virology, (1993 Jan) Vol. 67, No. 1, pp. 577-83. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Simian immunodeficiency virus** (**SIV**) infection of rhesus macaques is a model for **human immunodeficiency virus** (**HIV**) infection in humans. Inactivated and modified live whole-virus vaccines have provided limited protective immunity against **SIV** in rhesus macaques. Because of safety concerns in the use of inactivated and live whole-virus vaccines, we evaluated the protective immunity of vaccinia virus recombinants expressing the surface glycoprotein (gp130) of SIVmac and subunit preparations of gp130 expressed in mammalian cells (CHO). Three groups of animals were immunized with recombinant **SIV** gp130. The first group received **SIV** gp130 purified from genetically engineered CHO cells (cSIVgp130), the second group was vaccinated with recombinant vaccinia virus expressing SIVmac gp130 (vSIVgp130), and the third group was first primed with vSIVgp130 and then given a booster immunization with cSIVgp130. Although anti-gp130 binding antibodies were elicited in all three groups, neutralizing antibodies were transient or undetectable. None of the immunized animals resisted intravenous challenge with a low dose of cell-free virus. However, the group primed with vSIVgp130 and then boosted with cSIVgp130 had the lowest antigen load (p27) compared with the other groups. The results of these studies suggest that immunization of humans with **HIV** type 1 surface glycoprotein may not provide protective immunity against virus infection.

L5 ANSWER 24 OF 35 MEDLINE on STN
93059335. PubMed ID: 1433271. Characterization of group specific antibodies in primates: studies with **SIV envelope** in macaques. **Haigwood N L**; Misher L; Chin S M; Blair M; Planelles V; Scandella C J; Steimer K S; Gardner M B; Yilma T; Hirsch V M; +. (Chiron Corporation, Emeryville, CA 94608.) Journal of medical primatology, (1992 Feb-May) Vol. 21, No. 2-3, pp. 82-90. Journal code: 0320626. ISSN: 0047-2565. Pub. country: Denmark. Language: English.

AB Sera from **SIV**-infected macaques were found to contain antibodies that reacted with conformation-dependent, group-specific determinants on the **SIV envelope** protein gp130. These conformation-dependent antibodies exhibited virus neutralizing activity; their presence was associated with protection in vaccine studies. The properties of these antibodies are quite similar to those that have been identified in sera from **HIV**-infected human subjects. These data suggest that the **SIV envelope** gp130 remains a candidate for subunit vaccine studies.

L5 ANSWER 25 OF 35 MEDLINE on STN
92291314. PubMed ID: 1376330. Distinction of **human immunodeficiency virus** type 1 neutralization and infection enhancement by human monoclonal antibodies to glycoprotein 120. Takeda A; Robinson J E; Ho D D; Debouck C; **Haigwood N L**; Ennis F A. (Department of Medicine, University of Massachusetts Medical School, Worcester 01655.) The Journal of clinical investigation, (1992 Jun) Vol. 89, No. 6, pp. 1952-7. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language:

English.

AB There is increasing evidence that sera from **HIV**-1-infected individuals contain antibodies that enhance infection by **HIV**-1 in vitro. Previous work has demonstrated that complement receptors on T lymphoid cells and Fc receptors for IgG (Fc gamma R) on monocytic cells are required for enhanced infection by antibody-complexed **HIV**-1. Characterization of such infection-enhancing antibodies is essential because immunogenic epitopes which induce enhancing antibodies should be excluded from **HIV**-1 vaccines. This study was conducted to identify enhancing antibodies involved in Fc R-mediated enhancement of **HIV**-1 infection employing IgG human monoclonal antibodies (HMAbs) reactive against **gp120** of **HIV**-1, which were produced by B cell lines derived from an **HIV**-1-infected individual. A potent neutralizing HMAb N70-1.5e did not enhance infection by **HIV**-1 (IIIB and MN strains), whereas HMAb N70-2.3a mediated enhancement of **HIV**-1 infection, but had little neutralizing activity. A competition radio immunoassay demonstrated that the two antibodies bind to distinct epitopes. These results indicated that enhancing and neutralizing antibodies can be induced by different epitopes on **gp120**, suggesting the potential for development of safe vaccines against **HIV**-1 by exclusion of immunogenic epitopes for enhancing antibodies. We made attempts to identify the epitope on **gp120** that is recognized by the enhancing antibody N70-2.3a by using recombinant **HIV**-1 proteins and found that the antibody binds to a conformational site of nonvariable sequences in the carboxyl half (aa 272-509) of **gp120**.

L5 ANSWER 26 OF 35 MEDLINE on STN

92268867. PubMed ID: 1375277. Antibodies are produced to the variable regions of the external **envelope** glycoprotein of **human immunodeficiency virus** type 1 in chimpanzees infected with the virus and baboons immunized with a candidate recombinant vaccine. Stephens D M; Eichberg J W; **Haigwood N L**; Steimer K S; Davis D; Lachmann P J. (Molecular Immunopathology Unit, MRC Centre, Cambridge, U.K.) The Journal of general virology, (1992 May) Vol. 73 (Pt 5), pp. 1099-106. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Chimpanzees infected with **human immunodeficiency virus** type 1 produce antibodies against the variable regions of the external **envelope** glycoprotein **gp120**. All five variable regions contain an epitope which is recognized by at least one of five chimpanzee sera. Each of the sera recognized a different pattern of epitopes. It is suggested that this varying response contributes to the emergence of variant viruses in the host. In contrast with the variability of the chimpanzees' response to replicating virus, that of baboons to a candidate recombinant vaccine is more uniform. Baboons injected with recombinant **gp120** produced high levels of antibodies to epitopes within both the variable and conserved regions which coincided with epitopes previously shown to induce neutralizing antibodies.

L5 ANSWER 27 OF 35 MEDLINE on STN

92196080. PubMed ID: 1549578. Inactivated whole-virus vaccine derived from a proviral DNA clone of **simian immunodeficiency virus** induces high levels of neutralizing antibodies and confers protection against heterologous challenge. Johnson P R; Montefiori D C; Goldstein S; Hamm T E; Zhou J; Kitov S; **Haigwood N L**; Misher L; London W T; Gerin J L; +. (Department of Microbiology, Georgetown University, Rockville, MD 20852.) Proceedings of the National Academy of Sciences of the United States of America, (1992 Mar 15) Vol. 89, No. 6, pp. 2175-9. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We tested the ability of macaques vaccinated with inactivated whole **simian immunodeficiency virus** (**SIV**) to resist challenge with either homologous or heterologous cell-free uncloned **SIV** administered by the intravenous route. The vaccine virus was derived from a proviral DNA

clone and thus was considered genetically homogeneous. Sixteen macaques received either hepatitis B surface antigen (n = 6) or the inactivated whole-**SIV** vaccine (n = 10) at weeks 0, 4, and 49 of the study. All **SIV** vaccine recipients developed high levels of homologous and heterologous neutralizing antibodies in response to vaccination. At the time of challenge (week 53), vaccinees were further stratified to receive either homologous (n = 10) or heterologous (n = 6) uncloned live **SIV**. The **envelope** glycoproteins of the homologous and heterologous challenge viruses were 94% and 81% identical to the vaccine virus, respectively. Regardless of challenge inoculum, all vaccinees in the control group (hepatitis B surface antigen) became infected, whereas all **SIV** vaccinees were protected against detectable infection. These data support the concept that an efficacious vaccine for **HIV** might be possible, and suggest that genetic variation of **HIV** might not be an insurmountable obstacle for vaccine development.

L5 ANSWER 28 OF 35 MEDLINE on STN
92102765. PubMed ID: 1760229. Functional and immunological characterization of **SIV envelope** glycoprotein produced in genetically engineered mammalian cells. Planelles V; **Haigwood N L**; Marthas M L; Mann K A; Scandella C; Lidster W D; Shuster J R; Van Kuyk R; Marx P A; Gardner M B; +. (Department of Medical Pathology, University of California, Davis 95616.) AIDS research and human retroviruses, (1991 Nov) Vol. 7, No. 11, pp. 889-98. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Retroviral **envelope** glycoproteins interact with cell receptors and are targets for antiviral immune responses in infected hosts. Macaque **simian immunodeficiency virus** (SIVmac) is a T-lymphocytopathic lentivirus which causes an AIDS-like disease in rhesus macaques. The **envelope** gene of SIVmac encodes a precursor glycoprotein (**gp160**) which is cleaved into an external domain (gp130) and a transmembrane domain (gp32). To investigate the functional and immunological properties of the **SIV** external **envelope** glycoprotein, we have used genetically engineered mammalian cells to produce recombinant gp130 (rgp130). The rgp130 has the appropriate molecular weight, is glycosylated, and has native conformation as determined by binding to the cell receptor for **SIV**, the CD4 antigen. Rhesus macaques immunized with purified rgp130 formulated in muramyl dipeptide adjuvant generated high titers of antienvelope antibodies. Antibodies from these macaques were tested for in vitro virus neutralization; very low or undetectable levels of neutralization were observed. In contrast, neutralizing antibodies were readily detected in sera from goats immunized with rgp130. With respect to cell-mediated immunity, proliferative responses to rgp130 were demonstrated in peripheral blood monocyte cells (PBMC) from macaques immunized with the recombinant glycoprotein as well as in PBMC from **SIV**-infected animals. These results show that rgp130 is functional and immunogenic; the potential of rgp130 for protective immunization remains to be determined.

L5 ANSWER 29 OF 35 MEDLINE on STN
92085380. PubMed ID: 1727480. Native but not denatured recombinant **human immunodeficiency virus** type 1 **gp120** generates broad-spectrum neutralizing antibodies in baboons. **Haigwood N L**; Nara P L; Brooks E; Van Nest G A; Ott G; Higgins K W; Dunlop N; Scandella C J; Eichberg J W; Steimer K S. (Chiron Corporation, Emeryville, California 94608-2916.) Journal of virology, (1992 Jan) Vol. 66, No. 1, pp. 172-82. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The protection of individuals from **human immunodeficiency virus** type 1 (**HIV**-1) infection with an **envelope** subunit derived from a single isolate will require the presentation of conserved epitopes in **gp120**. The objective of the studies presented here was to test whether a native recombinant **gp120** (rgp120) immunogen would elicit responses to conserved

neutralization epitopes that are not present in a denatured recombinant **gp120** antigen from the same virus isolate. In a large study of 51 baboons, we have generated heterologous neutralizing activity with native, glycosylated rgp120SF2 but not with denatured, nonglycosylated **env** 2-3SF2. After repeated exposure to rgp120SF2 formulated with one of several adjuvants, virus isolates from the United States, the Caribbean, and Africa were neutralized. The timing of the immunization regimen and the choice of adjuvant affected the virus neutralization titers both quantitatively and qualitatively. These results suggest that vaccination with native, glycosylated rgp120 from a single virus isolate, **HIV**-SF2, may elicit a protective immune response effective against geographically and sequentially distinct **HIV**-1 isolates.

L5 ANSWER 30 OF 35 MEDLINE on STN
92022530. PubMed ID: 1718036. Neutralization of divergent **HIV**-1 isolates by conformation-dependent human antibodies to **Gp120**. Steimer K S; Scandella C J; Skiles P V; **Haigwood N L**. (Chiron Corporation, Emeryville, CA 94608.) Science, (1991 Oct 4) Vol. 254, No. 5028, pp. 105-8. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB The spectrum of **human immunodeficiency virus** type 1 (**HIV**-1) isolates neutralized by antibodies from **HIV**-1-infected humans is broader than the spectrum of isolates neutralized by sera from animals immunized with purified **gp120** subunits. This broader neutralization was due, in part, to the presence of antibodies to conserved **gp120** conformational epitopes. Purified conformation-dependent **gp120**-specific human antibodies neutralized a wider range of virus isolates than human antibodies directed to linear determinants in **gp120** and were also responsible for the majority of the **gp120**-specific CD4-blocking activity of **HIV**-1-infected human sera. A **gp120** subunit vaccine that effectively presents these conformation-dependent neutralization epitopes should protect against a broader range of **HIV**-1 variants than a vaccine that presents exclusively linear determinants.

L5 ANSWER 31 OF 35 MEDLINE on STN
92013142. PubMed ID: 1717587. N-glycosylation of **HIV-gp120** may constrain recognition by T lymphocytes. Botarelli P; Houlden B A; **Haigwood N L**; Servis C; Montagna D; Abrignani S. (Department of Allergy/Immunology, Ciba-Geigy Ltd, Basel, Switzerland.) Journal of immunology (Baltimore, Md. : 1950), (1991 Nov 1) Vol. 147, No. 9, pp. 3128-32. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The **HIV envelope** protein **gp120** is heavily glycosylated, having 55% of its molecular mass contributed by N-linked carbohydrates. We investigated the role of N-glycosylation in presentation of **HIV-gp120** to T cells. T cell clones obtained from humans immunized with a recombinant nonglycosylated form of **HIV-gp120** (**env** 2-3) were studied for their ability to recognize both **env** 2-3 and glycosylated **gp120**. We found that 20% of CD4+ T cell clones specific for **env** 2-3 fail to respond to glycosylated **gp120** of the same **HIV** isolate. Using synthetic peptides, we mapped one of the epitopes recognized by such clones to the sequence 292-300 (NESVAINCT), which contains two asparagines that are glycosylated in the native **gp120**. These findings suggest that N-linked carbohydrates within an epitope can function as hindering structures that limit Ag recognition by T lymphocytes.

L5 ANSWER 32 OF 35 MEDLINE on STN
91319560. PubMed ID: 1650459. Effect of intron A from human cytomegalovirus (Towne) immediate-early gene on heterologous expression in mammalian cells. Chapman B S; Thayer R M; Vincent K A; **Haigwood N L**. (Product Management and Regulatory Affairs, Chiron Corporation, Emeryville, CA 94608.) Nucleic acids research, (1991 Jul 25) Vol. 19, No.

14, pp. 3979-86. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A 2.4 kb fragment of hCMV (Towne strain), containing the 5' end of the major immediate-early gene, has been cloned, sequenced, and used to construct a series of mammalian cell expression plasmids. The effects of regulatory regions present on this fragment were assessed using human glycoproteins as reporter molecules. We compared secreted levels of Factor VIII, t-PA, and **HIV-1 envelope** glycoproteins in cells transfected with plasmids in which intron A of the immediate-early gene was present or absent. Secretion of several glycoproteins was significantly higher when cells were transfected with intron A-containing plasmids. Mutation of three basepairs in the strong nuclear factor 1 (NF1) binding site in intron A led to reduced transient expression levels, but not to the level observed in the absence of intron A. Reduced expression from NF1 mutant plasmids was roughly correlated with reduced binding in vitro of NF1 proteins to a synthetic oligonucleotide containing the mutation. The evidence indicates that sequences in intron A positively regulate expression from the hCMV immediate-early enhancer/promoter in transformed monkey kidney cells.

L5 ANSWER 33 OF 35 MEDLINE on STN

91250322. PubMed ID: 2040587. Antibody reactivity to deletion mutants of the **HIV**-1 SF2 **envelope**. Back N K; **Haigwood N L**; de Wolf F; de Jongh B M; Goudsmit J. (Department of Virology, Academic Medical Centre, Amsterdam, The Netherlands.) Intervirology, (1991) Vol. 32, No. 3, pp. 160-72. Journal code: 0364265. ISSN: 0300-5526. Pub. country: Switzerland. Language: English.

AB In **human immunodeficiency virus** type 1 (**HIV**-1) infected individuals, the antibody response to the external **envelope** (**gp120**) is associated with in vitro neutralization. To further characterize the anti-**gp120** response, we examined the IgG reactivity of 75 **HIV**-1-seroconverted and 200 **HIV**-1-seropositive individuals to deletion mutants of **gp120** in an enzyme immunoassay. We used yeast-derived, non-glycosylated recombinant **HIV**-1 SF2 **gp120** equivalent and-variants deleted in variable regions. We observed two distinctive response patterns: IgG non-responders (SF2-V3-restricted responders) and IgG responders to conserved regions of **gp120**. This divergence in response pattern occurred soon after gag/**env** **HIV**-1 antibody seroconversion and persisted in time within an individual. In addition, the SF2-V3-restricted responders had a higher frequency of **HIV**-1 core antigen positivity and **HIV**-1 core antibody negativity than the non-restricted responders. These results suggest that specific and persistent host antibody response patterns to **gp120** develop early in **HIV**-1 infection and that these patterns are associated with differences in **HIV**-1 expression.

L5 ANSWER 34 OF 35 MEDLINE on STN

90359271. PubMed ID: 2390335. Importance of hypervariable regions of **HIV**-1 **gp120** in the generation of virus neutralizing antibodies. **Haigwood N L**; Shuster J R; Moore G K; Lee H; Skiles P V; Higgins K W; Barr P J; George-Nascimento C; Steimer K S. (Chiron Research Laboratories, Chiron Corporation, Emeryville, CA 94608.) AIDS research and human retroviruses, (1990 Jul) Vol. 6, No. 7, pp. 855-69. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Variants of the **envelope** gene of the **HIV**-SF2 isolate of **HIV**-1 with deletions of one or more of the hypervariable domains of **gp120** were produced in genetically engineered yeast as nonglycosylated denatured polypeptide analogs of **gp120**. Purified antigens were used to immunize experimental animals to determine whether the removal of hypervariable regions from this type of **gp120** immunogen had any effect on (1) the ability of the antigen to elicit virus neutralizing antibodies; and (2) the isolate specificity of the neutralizing antibodies that were elicited.

The results of these studies demonstrate that, in addition to the previously identified V3 domain, at least two other hypervariable regions in **gp120** are capable of eliciting neutralizing antibodies in experimental animals. However, when all five of the hypervariable regions were deleted, the resulting antigen was no longer capable of eliciting neutralizing antibodies. Finally, the neutralizing antibodies elicited by all of these nonglycosylated antigens were effective against **HIV**-SF2, the isolate from which the antigens were derived, but were not able to neutralize two divergent isolates, **HIV**-BRU or **HIV**-Zr6.

L5 ANSWER 35 OF 35 MEDLINE on STN
90349569. PubMed ID: 1696717. Priming of CD4+ T cells specific for conserved regions of **human immunodeficiency virus** glycoprotein **gp120** in humans immunized with a recombinant **envelope** protein. Abrignani S; Montagna D; Jeannet M; Wintsch J; **Haigwood N L**; Shuster J R; Steimer K S; Cruchaud A; Staehelin T. (Department of Allergy/Immunology, CIBA-Geigy Ltd., Basel, Switzerland.) Proceedings of the National Academy of Sciences of the United States of America, (1990 Aug) Vol. 87, No. 16, pp. 6136-40. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB A nonglycosylated denatured form of **human immunodeficiency virus** (**HIV**) 1 glycoprotein **gp120** (**Env** 2-3), which does not bind to CD4, was used with muramyl tripeptide as adjuvant to immunize **HIV**-seronegative healthy volunteers. In all the volunteers, three 50-micrograms injections of **Env** 2-3 induced priming of CD4+ T cells specific for conserved regions of the native glycosylated **gp120**. Moreover, we found that several major histocompatibility complex class II (DR) alleles can function as restriction molecules for presentation of conserved epitopes of **gp120** to T cells, implying that a T-cell response to these epitopes can be obtained in a large fraction of the population. The possibility to prime CD4+ T cells specific for conserved epitopes of a **HIV** protein is particularly important in view of the lack of such cells in **HIV**-infected individuals and of a possible role that CD4+ T cells may play in the development of protective immunity against AIDS.

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L1           14 S E4

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L2           12 S E3

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L3           58 S E3-E5
L4           50 S L3 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L5           35 S L4 AND (ENV? OR GP160 OR GP120 OR GP41)

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                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    18.03     120.18

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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 21 Sep 2006 (20060921/PD)
FILE LAST UPDATED: 21 Sep 2006 (20060921/ED)
HIGHEST GRANTED PATENT NUMBER: US7111325
HIGHEST APPLICATION PUBLICATION NUMBER: US2006212984
CA INDEXING IS CURRENT THROUGH 21 Sep 2006 (20060921/UPCA)
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REVISED CLASS FIELDS (/NCL) LAST RELOADED: Jun 2006
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Jun 2006


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        521442 HUMAN
         25590 IMMUNODEFICIENCY
        105442 VIRUS
         18228 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
          3209 SIV
         17324 SIMIAN
         25590 IMMUNODEFICIENCY
        105442 VIRUS
          1819 SIMIAN IMMUNODEFICIENCY VIRUS
                 (SIMIAN(W)IMMUNODEFICIENCY(W)VIRUS)
L6       48156 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN IMMUNODEFI
               CIENCY VIRUS)

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          1695 GP160
          4834 GP120
          2530 GP41
L7       32395 L6 AND (ENV? OR GP160 OR GP120 OR GP41)

=> s l7 and glycosylat?
         40560 GLYCOSYLAT?
L8       12700 L7 AND GLYCOSYLAT?

=> s l8 and (N-linked or O-linked)
       1523489 N
        424632 LINKED
          8750 N-LINKED
                 (N(W)LINKED)
       1127066 O
        424632 LINKED
          6311 O-LINKED
                 (O(W)LINKED)
L9        4633 L8 AND (N-LINKED OR O-LINKED)

=> s l9 and (neutraliz?)
        198818 NEUTRALIZ?
L10       3810 L9 AND (NEUTRALIZ?)

=> s l10 and (env?/clm or gp160/clm or gp120/clm or gp41/clm)
        112721 ENV?/CLM
           200 GP160/CLM
           531 GP120/CLM
           235 GP41/CLM
L11        174 L10 AND (ENV?/CLM OR GP160/CLM OR GP120/CLM OR GP41/CLM)

=> s l11 and (N-linked/clm or O-linked/clm)
        433908 N/CLM
         82104 LINKED/CLM
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                  ((N(W)LINKED)/CLM)
         218845 O/CLM
          82104 LINKED/CLM
            212 O-LINKED/CLM
                  ((O(W)LINKED)/CLM)
L12          14 L11 AND (N-LINKED/CLM OR O-LINKED/CLM)

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L2           12 S E3

     FILE 'MEDLINE' ENTERED AT 20:54:28 ON 26 SEP 2006
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L3           58 S E3-E5
L4           50 S L3 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L5           35 S L4 AND (ENV? OR GP160 OR GP120 OR GP41)

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L8        12700 S L7 AND GLYCOSYLAT?
L9         4633 S L8 AND (N-LINKED OR O-LINKED)
L10        3810 S L9 AND (NEUTRALIZ?)
L11         174 S L10 AND (ENV?/CLM OR GP160/CLM OR GP120/CLM OR GP41/CLM)
L12          14 S L11 AND (N-LINKED/CLM OR O-LINKED/CLM)

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L13          12 L12 NOT L1

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L13  ANSWER 1 OF 12  USPATFULL on STN
2005:117715 Factor IX: remodeling and glycoconjugation of factor IX.
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    Bowe, Caryn, Doylestown, PA, UNITED STATES
    Hakes, David, Willow Grove, PA, UNITED STATES
    Chen, Xi, Lansdale, PA, UNITED STATES
    Neose Technologies, Inc. (U.S. corporation)
    US 2005100982 A1 20050512
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    US 2002-396594P  20020717 (60)
    US 2002-391777P  20020625 (60)
    US 2002-387292P  20020607 (60)
    US 2001-334301P  20011128 (60)
    US 2001-334233P  20011128 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1-112. (canceled)
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113. A cell-free, in vitro method of remodeling a Factor IX peptide, said peptide having the formula: ##STR143## wherein AA is a terminal or internal amino acid residue of said peptide; X_1-X_2 is a saccharide covalently linked to said AA, wherein X_1 is a first glycosyl residue; and X_2 is a second glycosyl residue covalently linked to X_1, wherein X_1 and X_2 are selected from monosaccharyl and oligosaccharyl residues; said method comprising: (a) removing X_2 or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said Factor IX peptide.

114. The method of claim 113, further comprising: (c) removing X_1, thereby exposing said AA; and (d) contacting said AA with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said AA, thereby remodeling said Factor IX peptide.

115. The method of claim 113, further comprising: (e) prior to step (b), removing a group added to said saccharide during post-translational modification.

116. The method of claim 115, wherein said group is a member selected from phosphate, sulfate, carboxylate and esters thereof.

117. The method of claim 113, wherein said peptide has the formula: ##STR144## wherein Z is a member selected from O, S, NH and a crosslinker.

118. The method of claim 113, wherein at least one of said glycosyl donors comprises a modifying group.

119. The method of claim 113, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

120. The method of claim 119, wherein said modifying group is a water soluble polymer.

121. The method of claim 120, wherein said water soluble polymer comprises poly(ethylene glycol).

122. The method of claim 120, wherein said water soluble polymer comprises methoxypoly(ethylene glycol).

123. The method of claim 121, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

124. A cell-free in vitro method of remodeling a Factor IX peptide, said peptide having the formula: ##STR145## wherein X_3, X_4, X_5, X_6, X_7, and X_{17} are independently selected monosaccharyl or oligosaccharyl residues; and a, b, c, d, e and x are independently selected from the integers 0, 1 and 2, with the proviso that at least one member selected from a, b, c, d, and e and x are 1 or 2; said method comprising: (a) removing at least one of X_3, X_4, X_5, X_6, X_7, or X_{17}, a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase

and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said Factor IX peptide.

125. A cell-free in vitro method of remodeling a Factor IX peptide, said peptide having the formula: ##STR146## wherein X_3, X_4, X_5, X_6, X_7, and X_{17} are independently selected monosaccharyl or oligosaccharyl residues; and a, b, c, d, e and x are independently selected from the integers 0, 1 and 2, with the proviso that at least one member selected from a, b, c, d, and e and x are 1 or 2; said method comprising contacting said glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said glycan, thereby remodeling said Factor IX peptide.

126. The method of claim 124, wherein said removing of step (a) produces a truncated glycan in which a, b, c, e and x are each 0.

127. The method of claim 124, wherein X_3, X_5, and X_7, are selected from the group consisting of $(mannose)_z$ and $(mannose)_z$--$(X_8)_y$ wherein X_8 is a glycosyl moiety selected from mono- and oligo-saccharides; y is an integer selected from 0 and 1; and z is an integer between 1 and 20, wherein when z is 3 or greater, (mannose), is selected from linear and branched structures.

128. The method of claim 124, wherein X_4 is selected from the group consisting of GlcNAc and xylose.

129. The method of claim 124, wherein X_3, X_5, and X_7 are $(mannose)_u$, wherein u is selected from the integers between 1 and 20, and when u is 3 or greater, $(mannose)_u$, is selected from linear and branched structures.

130. The method of claim 124, wherein at least one of said glycosyl donors comprises a modifying group.

131. The method of claim 130, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

132. The method of claim 131, wherein said modifying group is a water soluble polymer.

133. The method of claim 132, wherein said water soluble polymer comprises poly(ethylene glycol).

134. The method of claim 132, wherein said water soluble polymer comprises methoxypoly(ethylene glycol).

135. The method of claim 133, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

136. A cell-free in vitro method of remodeling a Factor IX peptide comprising a glycan having the formula: ##STR147## wherein r, s, and t are integers independently selected from 0 and 1, said method comprising: (a) contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said glycan, thereby remodeling said Factor IX peptide.

137. The method of claim 136, wherein at least one of said glycosyl donors comprises a modifying group.

138. The method of claim 137, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

139. The method of claim 138, wherein said modifying group is a water soluble polymer.

140. The method of claim 139, wherein said water soluble polymer comprises poly(ethylene glycol).

141. The method of claim 139, wherein said water soluble polymer comprises methoxypoly(ethylene glycol).

142. The method of claim 140, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

143. The method of claim 113, wherein said peptide has the formula: ##STR148## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1.

144. The method of claim 113, wherein said peptide has the formula: ##STR149## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1.

145. The method of claim 144, further wherein X_9 is a fucose residue and m is an integer of at least 1.

146. The method of claim 144, wherein said peptide comprises the formula ##STR150## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1, further wherein said peptide comprises at least two separate amino acid residues each having attached thereon at least one molecule of said saccharide.

147. The method of claim 113, wherein said peptide has the formula: ##STR151## wherein X_{11} and X_{12} are independently selected glycosyl moieties; and r and x are integers independently selected from 0 and 1.

148. The method of claim 147, wherein X_{11} and X_{12} are $(\text{mannose})_q$, wherein q is selected from the integers between 1 and 20, and when q is three or greater, $(\text{mannose})_q$ is selected from linear and branched structures.

149. The method of claim 113, wherein said peptide has the formula: ##STR152## wherein X_{13}, X_{14}, and X_{15} are independently selected glycosyl residues; and g, h, i, j, k, and p are independently selected from the integers 0 and 1, with the proviso that at least one of g, h, i, j, k and p is 1.

150. The method of claim 149, wherein X_{14} and X_{15} are members independently selected from GlcNAc and Sia; and i and k are independently selected from the integers 0 and 1, with the proviso that at least one of i and k is I and if k is 1, g, h and j are 0.

151. The method of claim 113, wherein said peptide has the formula:

##STR153## wherein X16 is a member selected from: ##STR154## wherein s and i are integers independently selected from 0 and 1.

152. The method of claim 113, wherein said removing utilizes a glycosidase.

153. A cell-free, in vitro method of remodeling a Factor IX peptide having the formula: ##STR155## wherein AA is a terminal or internal amino acid residue of said peptide; X1 is a glycosyl residue covalently linked to said AA, selected from monosaccharyl and oligosaccharyl residues; and u is an integer selected from 0 and 1, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, wherein said glycosyl donor comprises a modifying group, thereby remodeling said Factor IX peptide.

154. The method of claim 153, wherein said remodeled Factor IX peptide comprises at least one **N-linked** glycan and at least one **O-linked** glycan.

155. The method of claim 153, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

156. The method of claim 155, wherein said modifying group is a water soluble polymer.

157. The method of claim 156, wherein said water soluble polymer comprises poly(ethylene glycol).

158. The method of claim 157, wherein said water soluble polymer comprises methoxypoly(ethylene glycol).

159. The method of claim 157, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

160. A method of forming a covalent conjugate between a polymer and a **glycosylated** or non-**glycosylated** peptide, wherein said polymer is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said polymer, said method comprising: contacting said peptide with a mixture comprising a nucleotide sugar covalently linked to said polymer and a glycosyltransferase for which said nucleotide sugar is a substrate under conditions sufficient to form said conjugate, wherein said peptide is Factor IX.

161. The method of claim 160, wherein said polymer is a water-soluble polymer.

162. The method of claim 160, wherein said glycosyl linking group is covalently attached to a glycosyl residue covalently attached to said peptide.

163. The method of claim 160, wherein said glycosyl linking group is covalently attached to an amino acid residue of said peptide.

164. The method of claim 160, wherein said polymer comprises a member selected from the group consisting of a polyalkylene oxide and a polypeptide.

165. The method of claim 164, wherein said polyalkylene oxide is

poly(ethylene glycol).

166. The method of claim 165, wherein said polyalkylene oxide is methoxypoly(ethylene glycol).

167. The method of claim 165, wherein said poly(ethylene glycol) has a degree of polymerization of from about 1 to about 40,000.

168. The method of claim 165, wherein said poly(ethylene glycol) has a degree of polymerization of from about 1 to about 80,000, and further wherein said poly(ethylene glycol) is a branched structure.

169. The method of claim 165, wherein said poly(ethylene glycol) has a mass of from about 10 kDa to about 40 kDa.

170. The method of claim 165, wherein said poly(ethylene glycol) has a degree of polymerization of from about 1 to about 30,000, and further wherein said poly(ethylene glycol) is a linear structure.

171. The method of claim 167, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 5,000.

172. The method of claim 171, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 1,000.

173. The method of claim 160, wherein said glycosyltransferase is selected from the group consisting of sialyltransferase, galactosyltransferase, glucosyltransferase, GalNAc transferase, GlcNAc transferase, fucosyltransferase, mannosyltransferase, xylose transferase.

174. The method of claim 160, wherein said glycosyltransferase is recombinantly produced.

175. The method of claim 174, wherein said glycosyltransferase is a recombinant prokaryotic enzyme.

176. The method of claim 174, wherein said glycosyltransferase is a recombinant eukaryotic enzyme.

177. The method of claim 160, wherein said nucleotide sugar is selected from the group consisting of UDP-glycoside, CMP-glycoside, and GDP-glycoside.

178. The method of claim 177, wherein said nucleotide sugar is selected from the group consisting of UDP-galactose, UDP-galactosamine, UDP-glucose, UDP-glucosamine, UDP-N-acetylgalactosamine, UDP-N-acetylglucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid, CMP-NeuAc.

179. The method of claim 160, wherein said **glycosylated** peptide is partially deglycosylated prior to said contacting.

180. The method of claim 160, wherein said intact glycosyl linking group is a sialic acid residue.

181. The method of claim 160, wherein said method is performed in a cell-free **environment**.

182. The method of claim 160, wherein said covalent conjugate is isolated.

183. The method of claim 182, wherein said covalent conjugate is isolated by membrane filtration.

184. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR156## wherein AA is a terminal or internal amino acid residue of said peptide, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said amino acid residue, wherein said glycosyl donor comprises a modifying group, thereby remodeling said peptide, wherein said peptide is Factor IX.

185. A method for forming a conjugate between a Factor IX peptide and a modifying group, wherein said modifying group is covalently attached to said Factor IX peptide through an intact glycosyl linking group, said Factor IX peptide comprising a glycosyl residue having a formula which is a member selected from: ##STR157## wherein a, b, c, d, i, n, o, p, q, r, s, t, u, bb, cc, dd, ee, ff and gg are members independently selected from 0 and 1; e, f, g, h and aa are members independently selected from the integers from 0 to 6; j, k, I and m are members independently selected from the integers from 0 to 20; v, w, x, y and z are 0; R is a modifying group, a mannose or an oligomannose; said method comprising: (a) contacting said Factor IX peptide with a glycosyltransferase and a modified glycosyl donor, comprising a glycosyl moiety which is a substrate for said glycosyltransferase covalently linked to said modifying group, under conditions appropriate for the formation of said intact glycosyl linking group.

186. The method of claim 185, further comprising: (b) prior to step (a), contacting said Factor IX peptide with a sialidase under conditions appropriate to remove sialic acid from said Factor IX peptide.

187. The method of claim 185, further comprising: (c) contacting the product formed in step (a) with a sialyltransferase and a sialic acid donor under conditions appropriate to transfer sialic acid to said product.

188. The method of claim 186, further comprising: (d) contacting the product from step (b) with a galactosyltransferase and a galactose donor under conditions appropriate to transfer said galactose to said product.

189. The method of claim 188, further comprising: (e) contacting the product from step (d) with ST3Gal3 and a sialic acid donor under conditions appropriate to transfer sialic acid to said product.

190. The method of claim 185, further comprising: (d) contacting the product from step (a) with a moiety that reacts with said modifying group, thereby forming a conjugate between said intact glycosyl linking group and said moiety.

191. The method of claim 185, wherein said modifying group is a member selected from a polymer, a toxin, a radioisotope, a therapeutic moiety and a glycoconjugate.

192. The method of claim 185, wherein a, b, c, and d are 1; e, f, g and h are members independently selected from the integers from 1 to 4; aa, bb, cc, dd, ee, ff, j, k, l, m, i, n, o, p, q, r, s, t and u are members independently selected from 0 and 1; and v, w, x, y, z and gg are 0.

193. The method of claim 185, wherein a, b, c, d, n, q are independently selected from 0 and 1; aa, e, f, g and h are members

independently selected from the integers from 1 to 4; bb, cc, dd, ee, ff, j, k, l, m, i, o, p, r, s, t and u are members independently selected from 0 and 1; and v, w, x, y, z and gg are 0.

194. The method of claim 185, wherein a, b, c, d, n, bb, cc, dd and ff are 1; e, f, g, h and aa are members independently selected from the integers from 1 to 4; q, ee, i, j, k, l, m, o, p, r, s, t and u are members independently selected from 0 and 1; and v, w, x, y, z and gg are 0.

195. The method of claim 185, wherein a, b, c, d and q are 1; e, f, g and h are members independently selected from the integers from 1 to 4; aa, bb, cc, dd, ee, ff, j, k, l, m, i, n, o, p, r, s, t and u are members independently selected from 0 and 1; and v, w, x, y, z and gg are 0.

196. The method of claim 185, wherein a, b, c, d, q, bb, cc, dd and ff are 1; aa, e, f, g and h are members independently selected from the integers from 1 to 4; ee, i, j, k, l, m, o, p, r, s, t and u are members independently selected from 0 and 1; and v, w, x, y, z and gg are 0.

197. A method of treating a mammal having hemophilia B, said method comprising administering to said mammal a Factor IX peptide having one or more glycans having a glycoconjugate molecule attached to said peptide.

198. The method of claim 197, wherein said glyconjugate is poly(ethylene glycol).

199. The method of claim 197, wherein said mammal is a human.

L13 ANSWER 2 OF 12 USPATFULL on STN
2005:36910 Interleukin-2:remodeling and glycoconjugation of interleukin-2.
DeFrees, Shawn, North Wales, PA, UNITED STATES
Zopf, David, Wayne, PA, UNITED STATES
Bayer, Robert, San Diego, CA, UNITED STATES
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Neose Technologies, Inc. (U.S. corporation)
US 2005031584 A1 20050210
APPLICATION: US 2003-410980 A1 20030409 (10)
PRIORITY: US 2002-407527P 20020828 (60)
US 2002-404249P 20020816 (60)
US 2002-396594P 20020717 (60)
US 2002-391777P 20020625 (60)
US 2002-387292P 20020607 (60)
US 2001-334301P 20011128 (60)
US 2001-334233P 20011128 (60)
US 2001-344692P 20011019 (60)
US 2001-328523P 20011010 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A cell-free, in vitro method of remodeling an interleukin-2 (IL-2) peptide, said peptide having the formula: ##STR143## wherein AA is a terminal or internal amino acid residue of said peptide; X_1-X_2 is a saccharide covalently linked to said AA, wherein X_1 is a first glycosyl residue; and X_2 is a second glycosyl residue covalently linked to X_1, wherein X_1 and X_2 are

selected from monosaccharyl and oligosaccharyl residues; said method comprising: (a) removing X_2 or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said IL-2 peptide.

2. The method of claim 1, further comprising: (c) removing X_1, thereby exposing said AA; and (d) contacting said AA with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said AA, thereby remodeling said IL-2 peptide.

3. The method of claim 1, further comprising: (e) prior to step (b), removing a group added to said saccharide during post-translational modification.

4. The method of claim 3, wherein said group is a member selected from phosphate, sulfate, carboxylate and esters thereof.

5. The method of claim 1, wherein said peptide has the formula: ##STR144## wherein Z is a member selected from O, S, NH or a crosslinker.

6. The method of claim 1, wherein at least one of said glycosyl donors comprises a modifying group.

7. The method of claim 1, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

8. The method of claim 7, wherein said modifying group is a water soluble polymer.

9. The method of claim 8, wherein said water soluble polymer comprises poly(ethylene glycol).

10. The method of claim 9, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

11. A cell-free in vitro method of remodeling an IL-2 peptide, said peptide having the formula: ##STR145## wherein X_3, X_4, X_5, X_6, X_7, and X_{17} are independently selected monosaccharyl or oligosaccharyl residues; and a, b, c, d, e, and x are independently selected from the integers 0, 1 and 2, with the proviso that at least one member selected from a, b, c, d, and e and x are 1 or 2; said method comprising: (a) removing at least one of X_3, X_4, X_5, X_6, X_7, or X_{17}, a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said IL-2 peptide.

12. The method of claim 11, wherein said removing of step (a) produces a truncated glycan in which a, b, c, e and x are each 0.

13. The method of claim 11, wherein X_3, X_5, and X_7 are selected from the group consisting of $(\text{mannose})_z$ and $(\text{mannose})_n\text{-}(X_8)_y$ wherein X_8 is a glycosyl moiety

selected from mono- and oligo-saccharides; y is an integer selected from 0 and 1; and z is an integer between 1 and 20, wherein when z is 3 or greater, (mannose)z is selected from linear and branched structures.

14. The method of claim 11, wherein X_4 is selected from the group consisting of GlcNAc and xylose.

15. The method of claim 11, wherein X_3, X_5, and X_7 are (mannose)u, wherein u is selected from the integers between 1 and 20, and when u is 3 or greater, (mannose)u is selected from linear and branched structures.

16. The method of claim 11, wherein at least one of said glycosyl donors comprises a modifying group.

17. The method of claim 16, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

18. The method of claim 17 wherein said modifying group is a water soluble polymer.

19. The method of claim 18, wherein said water soluble polymer comprises poly(ethylene glycol).

20. The method of claim 19, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

21. A cell-free in vitro method of remodeling an IL-2 peptide comprising a glycan having the formula: ##STR146## wherein r, s, and t are integers independently selected from 0 and 1, said method comprising: (a) contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said glycan, thereby remodeling said IL-2 peptide.

22. The method of claim 21, wherein at least one of said glycosyl donors comprises a modifying group.

23. The method of claim 22, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

24. The method of claim 23 wherein said modifying group is a water soluble polymer.

25. The method of claim 24, wherein said water soluble polymer comprises poly(ethylene glycol).

26. The method of claim 25, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

27. The method of claim 1, wherein said peptide has the formula: ##STR147## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1.

28. The method of claim 1, wherein said peptide has the formula: ##STR148## wherein X_{11} and X_{12} are independently selected

glycosyl moieties; and r and x are integers independently selected from 0 and 1.

29. The method of claim 28, wherein X_{11} and X_{12} are (mannose)$_q$, wherein q is selected from the integers between 1 and 20, and when q is three or greater, (mannose)$_q$ is selected from linear and branched structures.

30. The method of claim 1, wherein said peptide has the formula: ##STR149## wherein X_{13}, X_{14}, and X_{15} are independently selected glycosyl residues; and g, h, i, j, k, and p are independently selected from the integers 0 and 1, with the proviso that at least one of g, h, i, j, k and p is 1.

31. The method of claim 30, wherein X_{14} and X_{15} are members independently selected from GlcNAc and Sia; and i and k are independently selected from the integers 0 and 1, with the proviso that at least one of i and k is 1 and if k is 1, g, h, and j are 0.

32. The method of claim 1, wherein said peptide has the formula: ##STR150## wherein X_{16} is a member selected from: ##STR151## wherein s and i are integers independently selected from 0 and 1.

33. The method of claim 1, wherein said removing utilizes a glycosidase.

34. A cell-free, in vitro method of remodeling an IL-2 peptide having the formula: ##STR152## wherein AA is a terminal or internal amino acid residue of said peptide; X_1 is a glycosyl residue covalently linked to said AA, selected from monosaccharyl and oligosaccharyl residues; and u is an integer selected from 0 and 1, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, wherein said glycosyl donor comprises a modifying group, thereby remodeling said IL-2 peptide.

35. The method of claim 34, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

36. The method of claim 35 wherein said modifying group is a water soluble polymer.

37. The method of claim 36, wherein said water soluble polymer comprises poly(ethylene glycol).

38. The method of claim 37, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

39. A covalent conjugate between a IL-2 peptide and a modifying group that alters a property of said peptide, wherein said modifying group is covalently attached to said peptide at a preselected glycosyl or amino acid residue of said peptide via an intact glycosyl linking group.

40. The covalent conjugate of claim 39, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

41. The covalent conjugate of claim 39, wherein said modifying group and an intact glycosyl linking group precursor are linked as a covalently

attached unit to said peptide via the action of an enzyme, said enzyme converting said precursor to said intact glycosyl linking group, thereby forming said conjugate.

42. The covalent conjugate of claim 39 comprising: a first modifying group covalently linked to a first residue of said peptide via a first intact glycosyl linking group, and a second glycosyl linking group linked to a second residue of said peptide via a second intact glycosyl linking group.

43. The covalent conjugate of claim 42, wherein said first residue and said second residue are structurally identical.

44. The covalent conjugate of claim 42, wherein said first residue and said second residue have different structures.

45. The covalent conjugate of claim 42 wherein said first residue and said second residue are glycosyl residues.

46. The covalent conjugate of claim 42, wherein said first residue and said second residue are amino acid residues.

47. The covalent conjugate of claim 39, wherein said peptide is remodeled prior to forming said conjugate.

48. The covalent conjugate of claim 47, wherein the remodeled peptide is remodeled to introduce an acceptor moiety for said intact glycosyl linking group.

49. The covalent conjugate of claim 39, wherein said modifying group is a water-soluble polymer.

50. The covalent conjugate of claim 49, wherein said water-soluble polymer comprises poly(ethylene glycol).

51. The covalent conjugate of claim 39, wherein said intact glycosyl linking unit is a member selected from the group consisting of a sialic acid residue, a Gal residue, a GlcNAc residue and a GalNAc residue.

52. The covalent conjugate of claim 50, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

53. A method of forming a covalent conjugate between a polymer and a **glycosylated** or non-**glycosylated** peptide, wherein said polymer is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said polymer, said method comprising: contacting said peptide with a mixture comprising a nucleotide sugar covalently linked to said polymer and a glycosyltransferase for which said nucleotide sugar is a substrate under conditions sufficient to form said conjugate, wherein said peptide is IL-2.

54. The method of claim 53, wherein said polymer is a water-soluble polymer.

55. The method of claim 53, wherein said glycosyl linking group is covalently attached to a glycosyl residue covalently attached to said peptide.

56. The method of claim 53, wherein said glycosyl linking group is covalently attached to an amino acid residue of said peptide.

57. The method of claim 53, wherein said polymer comprises a member selected from the group consisting of a polyalkylene oxide and a polypeptide.

58. The method of claim 57, wherein said polyalkylene oxide is poly(ethylene glycol).

59. The method of claim 58, wherein said poly(ethylene glycol) has a degree of polymerization of from about 1 to about 20,000.

60. The method of claim 59, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 5,000.

61. The method of claim 60, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 1,000.

62. The method of claim 53, wherein said glycosyltransferase is selected from the group consisting of sialyltransferase, galactosyltransferase, glucosyltransferase, GalNAc transferase, GlcNAc transferase, fucosyltransferase, and mannosyltransferase.

63. The method of claim 53, wherein said glycosyltransferase is recombinantly produced.

64. The method of claim 63, wherein said glycosyltransferase is a recombinant prokaryotic enzyme.

65. The method of claim 63, wherein said glycosyltransferase is a recombinant eukaryotic enzyme.

66. The method of claim 53, wherein said nucleotide sugar is selected from the group consisting of UDP-glycoside, CMP-glycoside, and GDP-glycoside.

67. The method of claim 66, wherein said nucleotide sugar is selected from the group consisting of UDP-galactose, UDP-galactosamine, UDP-glucose, UDP-glucosamine, UDP-N-acetylgalactosamine, UDP-N-acetylglucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid, CMP-NeuAc.

68. The method of claim 53, wherein said **glycosylated** peptide is partially deglycosylated prior to said contacting.

69. The method of claim 53, wherein said intact glycosyl linking group is a sialic acid residue.

70. The method of claim 53, wherein said method is performed in a cell-free **environment**.

71. The method of claim 53, wherein said covalent conjugate is isolated.

72. The method of claim 71, wherein said covalent conjugate is isolated by membrane filtration.

73. A composition for forming a conjugate between a peptide and a modified sugar, said composition comprising: an admixture of a modified sugar, a glycosyltransferase, and a peptide acceptor substrate, wherein said modified sugar has covalently attached thereto a member selected from a polymer, a therapeutic moiety and a biomolecule, wherein said peptide is IL-2.

74. An IL-2 peptide remodeled by the method of claim 1.

75. A pharmaceutical composition comprising the IL-2 peptide of claim 74.

76. An IL-2 peptide remodeled by the method of claim 11.

77. A pharmaceutical composition comprising the IL-2 peptide of claim 76.

78. An IL-2 peptide remodeled by the method of claim 21.

79. A pharmaceutical composition comprising the IL-2 peptide of claim 78.

80. An IL-2 peptide remodeled by the method of claim 27.

81. A pharmaceutical composition comprising the IL-2 peptide of claim 80.

82. An IL-2 peptide remodeled by the method of claim 28.

83. A pharmaceutical composition comprising the IL-2 peptide of claim 82.

84. An IL-2 peptide remodeled by the method of claim 34.

85. A pharmaceutical composition comprising the IL-2 peptide of claim 84.

86. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR153## wherein AA is a terminal or internal amino acid residue of said peptide, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said amino acid residue, wherein said glycosyl donor comprises a modifying group, thereby remodeling said peptide, wherein said peptide is IL-2.

87. A method of forming a conjugate between a IL-2 peptide and a modifying group, wherein said modifying group is covalently attached to said IL-2 peptide through an intact glycosyl linking group, said IL-2 peptide comprising a glycosyl residue having a formula which is a member selected from: ##STR154## wherein a, b, c, and e are members independently selected from 0 and 1; d is 0; and R is a modifying group, said method comprising: (a) contacting said IL-2 peptide with a glycosyltransferase and a modified glycosyl donor, comprising a glycosyl moiety which is a substrate for said glycosyltransferase covalently linked to said modifying group, under conditions appropriate for the formation of said intact glycosyl linking group.

88. The method of claim 87, further comprising: (b) prior to step (a), contacting said 1L-2 peptide with a sialidase under conditions appropriate to remove sialic acid from said IL-2 peptide.

89. The method of claim 87, further comprising: (c) prior to step (a), contacting said IL-2 peptide with an endo-N-acetylgalactosaminidase operating synthetically under conditions appropriate to add a GalNAc to said IL-2 peptide.

90. The method of claim 87, further comprising: (d) contacting the product from step (a) with a moiety that reacts with said modifying

group, thereby forming a conjugate between said intact glycosyl linking group and said moiety.

91. The method of claim 87, further comprising: (e) prior to step (a), contacting said IL-2 peptide with N-acetylgalactosamine transferase and a GalNAc donor under conditions appropriate to transfer GalNAc to said IL-2 peptide.

92. The method of claim 87, further comprising (f) prior to step (a) contacting said IL-2 peptide with galactosyltransferase and a galactose donor under conditions appropriate to transfer galactose to said IL-2 peptide.

93. The method of claim 87, wherein said modifying group is a member selected from a polymer, a toxin, a radioisotope, a therapeutic moiety and a glycoconjugate.

94. The method of claim 87, wherein a and e are members independently selected from 0 and 1; and b, c, and d are 0.

95. The method of claim 87, wherein a, b, c, d, and e are 0.

96. An IL-2 peptide conjugate formed by the method of claim 87.

97. An IL-2 peptide comprising one or more glycans, having a glycoconjugate molecule covalently attached to said peptide.

98. The IL-2 peptide of claim 97, wherein said one or more glycans is a monoantennary glycan.

99. The IL-2 peptide of claim 97, wherein said one or more glycans is a biantennary glycan.

100. The IL-2 peptide of claim 97, wherein said one or more glycans is a triantennary glycan.

101. The IL-2 peptide of claim 97, wherein said one or more glycans is at least a triantennary glycan.

102. The IL-2 peptide of claim 97, wherein said one or more glycans comprises at least two glycans comprising a mixture of mono or multiantennary glycans.

103. The IL-2 peptide of claim 97, wherein said one or more glycans is selected from an **N-linked** glycan and an **O-linked** glycan.

104. The IL-2 peptide of claim 97, wherein said one or more glycans is at least two glycans selected from an **N-linked** and an **O-linked** glycan.

105. The IL-0.2 peptide of claim 97, wherein said peptide is expressed in a cell selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

106. The IL-2 peptide of claim 105, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell and a yeast cell.

117. A method of stimulating cytotoxic T cells in a mammal, said method comprising administering to said mammal an IL-2 peptide having one or more glycans having a glycoconjugate molecule attached to said peptide.

118. The method of claim 117, wherein said glycoconjugate is

poly(ethylene glycol).

119. The method of claim 117, wherein said mammal is a human.

120. The method of claim 117, wherein said human has cancer.

121. The method of claim 117, wherein said human has Acquired Immunodeficiency Disease.

L13 ANSWER 3 OF 12 USPATFULL on STN
2004:184970 Glycoconjugation methods and proteins/peptides produced by the methods.
DeFrees, Shawn, North Wales, PA, UNITED STATES
Zopf, David, Wayne, PA, UNITED STATES
Bayer, Robert, San Diego, CA, UNITED STATES
Bowe, Caryn, Doylestown, PA, UNITED STATES
Hakes, David, Willow Grove, PA, UNITED STATES
Chen, Xi, Lansdale, PA, UNITED STATES
Neose Technologies, Inc. (U.S. corporation)
US 2004142856 A1 20040722
APPLICATION: US 2003-410913 A1 20030409 (10)
PRIORITY: US 2002-407527P 20020828 (60)
US 2002-407527P 20020828 (60)
US 2002-404249P 20020816 (60)
US 2002-396594P 20020717 (60)
US 2002-391777P 20020625 (60)
US 2002-387292P 20020607 (60)
US 2001-334301P 20011128 (60)
US 2001-334233P 20011128 (60)
US 2001-334692P 20011121 (60)
US 2001-328523P 20011010 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR152## wherein AA is a terminal or internal amino acid residue of said peptide; X1-X2 is a saccharide covalently linked to said AA, wherein X1 is a first glycosyl residue; and X2 is a second glycosyl residue covalently linked to X1, wherein X1 and X2 are selected from monosaccharyl and oligosaccharyl residues; said method comprising: (a) removing X2 or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said peptide.

2. The method of claim 1, further comprising: (c) removing X1, thereby exposing said AA; and (d) contacting said AA with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said AA, thereby remodeling said peptide.

3. The method of claim 1, further comprising: (e) prior to step (b), removing a group added to said saccharide during post-translational modification.

4. The method of claim 3, wherein said group is a member selected from phosphate, sulfate, carboxylate and esters thereof.

5. The method of claim 1, wherein said peptide has the formula:

##STR153## wherein Z is a member selected from O, S NH, and a crosslinker.

6. The method of claim 1, wherein at least one of said glycosyl donors comprises a modifying group.

7. The method of claim 6, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

8. The method of claim 7, wherein said modifying group is a water soluble polymer.

9. A cell-free in vitro method of remodeling a peptide having the formula: ##STR154## wherein X_3, X_4, X_5, X_6, X_7 and X_{17} are independently selected monosaccharyl or oligosaccharyl residues; and a, b, c, d, e, and x are independently selected from the integers 0, 1 and 2, with the proviso that at least one member selected from a, b, c, d, e, and x is 1 or 2; said method comprising: (a) removing at least one of X_3, X_4, X_5, X_6, X_7 or X_{17}, or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said peptide.

10. The method of claim 9, wherein said removing of step (a) produces a truncated glycan in which a, b, c, e and x are each 0.

11. The method of claim 9, wherein X_3, X_5 and X_7 are selected from the group consisting of $(mannose)_z$ and $(mannose)_z$-$(X_8)_y$ wherein X_8 is a glycosyl moiety selected from mono- and oligo-saccharides; y is an integer selected from 0 and 1; and z is an integer between 1 and 20, wherein when z is 3 or greater, $(mannose)_z$ is selected from linear and branched structures.

12. The method of claim 9, wherein X_4 is selected from the group consisting of GlcNAc and xylose.

13. The method of claim 9, wherein X_3, X_5 and X_7 are $(mannose)_u$, wherein u is selected from the integers between 1 and 20, and when u is 3 or greater, $(mannose)_u$ is selected from linear and branched structures.

14. The method of claim 9, wherein at least one of said glycosyl donors comprises a modifying group.

15. The method of claim 14, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety and a peptide.

16. The method of claim 15 wherein said modifying group is a water soluble polymer.

17. A cell-free in vitro method of remodeling a peptide comprising a glycan having the formula: ##STR155## wherein r, s, and t are integers independently selected from 0 and 1, said method comprising: (a) contacting said peptide with at least one glycosyltransferase and at

least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said glycan, thereby remodeling said peptide.

18. The method of claim 17, wherein at least one of said glycosyl donors comprises a modifying group.

19. The method of claim 17, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety and a peptide.

20. The method of claim 19 wherein said modifying group is a water soluble polymer.

21. The method of claim 1, wherein said peptide has the formula: ##STR156## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1.

22. The method of claim 1, wherein said peptide has the formula: ##STR157## wherein X_{11} and X_{12} are independently selected glycosyl moieties; and r and x are integers independently selected from 0 and 1.

23. The method of claim 22, wherein X_{11} and X_{12} are $(\text{mannose})_q$, wherein q is selected from the integers between 1 and 20, and when q is three or greater, $(\text{mannose})_q$ is selected from linear and branched structures.

24. The method of claim 21, wherein said peptide has the formula: ##STR158## wherein X_{13}, X_{14}, and X_{15} are independently selected glycosyl residues; and g, h, i, j, k, and p are independently selected from the integers 0 and 1, with the proviso that at least one of g, h, i, j, k and p is 1.

25. The method of claim 24, wherein X_{14} and X_{15} are members independently selected from GlcNAc and Sia; and i and k are independently selected from the integers 0 and 1, with the proviso that at least one of i and k is 1, and if k is 1, g, h, and j are 0.

26. The method of claim 1, wherein said peptide has the formula: ##STR159## wherein X_{16} is a member selected from: ##STR160## wherein s, u and i are independently selected from the integers 0 and 1.

27. The method of claim 1, wherein said removing utilizes a glycosidase.

28. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR161## wherein AA is a terminal or internal amino acid residue of said peptide; X_1 is a glycosyl residue covalently linked to said AA, selected from monosaccharyl and oligosaccharyl residues; and u is an integer selected from 0 and 1, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, wherein said glycosyl donor comprises a modifying group, thereby remodeling said peptide.

29. The method of claim 28, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a

peptide.

30. The method of claim 29 wherein said modifying group is a water soluble polymer.

31. A covalent conjugate between a peptide and a modifying group that alters a property of said peptide, wherein said modifying group is covalently attached to said peptide at a preselected glycosyl or amino acid residue of said peptide via an intact glycosyl linking group.

32. The covalent conjugate of claim 31, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

33. The covalent conjugate of claim 31, wherein said modifying group and an intact glycosyl linking group precursor are linked as a covalently attached unit to said peptide via the action of an enzyme, said enzyme converting said precursor to said intact glycosyl linking group, thereby forming said conjugate.

34. The covalent conjugate of claim 31 comprising: a first modifying group covalently linked to a first residue of said peptide via a first intact glycosyl linking group, and a second glycosyl linking group linked to a second residue of said peptide via a second intact glycosyl linking group.

35. The covalent conjugate of claim 34, wherein said first residue and said second residue are structurally identical.

36. The covalent conjugate of claim 34, wherein said first residue and said second residue have different structures.

37. The covalent conjugate of claim 34 wherein said first residue and said second residue are glycosyl residues.

38. The covalent conjugate of claim 34, wherein said first residue and said second residue are amino acid residues.

39. The covalent conjugate of claim 31, wherein said peptide is remodeled prior to forming said conjugate.

40. The covalent conjugate of claim 39, wherein the remodeled peptide is remodeled to introduce an acceptor moiety for said intact glycosyl linking group.

41. The covalent conjugate of claim 31, wherein said modifying group is a water-soluble polymer.

42. The covalent conjugate of claim 31, wherein said intact glycosyl linking unit is a member selected from the group consisting of a sialic acid residue, a Gal residue, a GlcNAc residue and a GalNAc residue.

43. A method of forming a covalent conjugate between a polymer and a **glycosylated** or non-**glycosylated** peptide, wherein said polymer is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said polymer, said method comprising: contacting said peptide with a mixture comprising a nucleotide sugar covalently linked to said polymer and a glycosyltransferase for which said nucleotide sugar is a substrate under conditions sufficient to form said conjugate.

44. The method of claim 43, wherein said polymer is a water-soluble polymer.

45. The method of claim 43, wherein said glycosyl linking group is covalently attached to a glycosyl residue covalently attached to said peptide.

46. The method of claim 43, wherein said glycosyl linking group is covalently attached to an amino acid residue of said peptide.

47. The method of claim 43, wherein said polymer comprises a member selected from the group consisting of a polyalkylene oxide and a polypeptide.

48. The method of claim 43, wherein said glycosyltransferase is selected from the group consisting of sialyltransferase, galactosyltransferase, glucosyltransferase, GalNAc transferase, GlcNAc transferase, fucosyltransferase, and mannosyltransferase.

49. The method of claim 43, wherein said glycosyltransferase is recombinantly produced.

50. The method of claim 49, wherein said glycosyltransferase is a recombinant prokaryotic enzyme.

51. The method of claim 49, wherein said glycosyltransferase is a recombinant eukaryotic enzyme.

52. The method of claim 43, wherein said nucleotide sugar is selected from the group consisting of UDP-glycoside, CMP-glycoside, and GDP-glycoside.

53. The method of claim 52, wherein said nucleotide sugar is selected from the group consisting of UDP-galactose, UDP-galactosamine, UDP-glucose, UDP-glucosamine, UDP-N-acetylgalactosamine, UDP-N-acetylglucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid, CMP-NeuAc.

54. The method of claim 43, wherein said peptide is a therapeutic agent.

55. The method of claim 43, wherein said **glycosylated** peptide is partially deglycosylated prior to said contacting.

56. The method of claim 43, wherein said intact glycosyl linking group is a sialic acid residue.

57. The method of claim 43, wherein said method is performed in a cell-free **environment**.

58. The method of claim 43, wherein said covalent conjugate is isolated.

59. The method of claim 58, wherein said covalent conjugate is isolated by membrane filtration.

60. A method of forming a covalent conjugate between a first **glycosylated** or non-**glycosylated** peptide, and a second **glycosylated** or non-**glycosylated** peptide cojoined by a linker moiety, wherein said linker moiety is conjugated to said first peptide via a first intact glycosyl linking group interposed between and covalently linked to both said first peptide and said linker moiety, and said linker moiety is conjugated to said second peptide via a second intact glycosyl linking group interposed between and covalently linked

to both said second peptide and said linker moiety; said method comprising: (a) contacting said first peptide with a derivative of said linker moiety precursor comprising a precursor of said first intact glycosyl linking group and a precursor of said second intact glycosyl linking group; (b) contacting the mixture from (a) with a glycosyl transferase for which said precursor of said first glycosyl linking group is a substrate, under conditions sufficient to convert said precursor of said first intact glycosyl linking group into said first intact glycosyl linking group, thereby forming a first conjugate between said linker moiety precursor and said first peptide; (c) contacting said first conjugate with said second peptide and a glycosyltransferase for which said precursor of said second intact glycosyl group is a substrate under conditions sufficient to convert said precursor of said second intact glycosyl linking group into said second glycosyl linking group, thereby forming said conjugate between said linker moiety and said first **glycosylated** or non-**glycosylated** peptide, and said second **glycosylated** or non-**glycosylated** peptide.

61. The method of claim 60, wherein said linker moiety comprises a water-soluble polymer.

62. A method of forming a covalent conjugate between a first **glycosylated** or non-**glycosylated** peptide, and a second **glycosylated** or non-**glycosylated** peptide cojoined by a linker moiety, wherein said linker moiety is covalently conjugated to said first peptide, and said linker moiety is conjugated to said second peptide via an intact glycosyl linking group interposed between and covalently linked to both said second peptide and said linker moiety, said method comprising: (a) contacting said first peptide with an activated derivative of said linker moiety comprising; a reactive functional group of reactivity complementary to a residue on said first peptide, and a precursor of said intact glycosyl linking group, under conditions sufficient to form a covalent bond between said reactive functional group and said residue, thereby forming a first conjugate; and (b) contacting said first conjugate with said second peptide and a glycosyltransferase for which said precursor of said intact glycosyl linking group is a substrate, under conditions sufficient to convert said precursor of said intact glycosyl linking group into said intact glycosyl linking group, thereby forming said conjugate between said first **glycosylated** or non-**glycosylated** peptide, and said second **glycosylated** or non-**glycosylated** peptide cojoined by said linker moiety.

63. The method of claim 22, wherein said linker moiety comprises a water-soluble polymer.

64. A pharmaceutical composition comprising a pharmaceutically acceptable diluent and a covalent conjugate between a polymer and a **glycosylated** or non-**glycosylated** peptide, wherein said polymer is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said polymer.

65. A composition for forming a conjugate between a peptide and a modified sugar, said composition comprising: an admixture of a modified sugar, a glycosyltransferase, and a peptide acceptor substrate, wherein said modified sugar has covalently attached thereto a member selected from a polymer, a therapeutic moiety and a biomolecule.

66. A peptide remodeled by the method of claim 1.

67. A pharmaceutical composition comprising the peptide of claim 66.

68. A peptide remodeled by the method of claim 9.

69. A pharmaceutical composition comprising the peptide of claim 68.

70. A peptide remodeled by the method of claim 17.

71. A pharmaceutical composition comprising the peptide of claim 70.

72. A peptide remodeled by the method of claim 21.

73. A pharmaceutical composition comprising the peptide of claim 72.

74. A peptide remodeled by the method of claim 28.

75. A pharmaceutical composition comprising the peptide of claim 74.

76. A compound having the formula: ##STR162## wherein MS is a modified sugar comprising a sugar covalently bonded to a modifying group; Nu is a nucleoside; and b is an integer from 0 to 2.

77. The compound according to claim 76, having the formula: ##STR163## wherein X, Y, Z, A and B are members independently selected from S, O and NH; R21, R22, R23, R24, and R25 members independently selected from H and a polymer; R26 is a member selected from H, OH, and a polymer; R27 is a member selected from COO- and Na+; Nu is a nucleoside; a is an integer from 1 to 3 and i+is a salt.

78. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR164## wherein AA is a terminal or internal amino acid residue of said peptide, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said amino acid residue, wherein said glycosyl donor comprises a modifying group, thereby remodeling said peptide.

79. A peptide comprising one or more glycans, having a glycoconjugate molecule covalently attached to said peptide.

80. The peptide of claim 79, wherein said one or more glycans is a monoantennary glycan.

81. The peptide of claim 79, wherein said one or more glycans is a biantennary glycan.

82. The peptide of claim 79, wherein said one or more glycans is a triantennary glycan.

83. The peptide of claim 79, wherein said one or more glycans is at least a triantennary glycan.

84. The peptide of claim 79, wherein said one or more glycans comprises at least two glycans comprising a mixture of mono and multiantennary glycans.

85. The peptide of claim 79, wherein said one or more glycans is selected from an **N-linked** glycan and an **O-linked** glycan.

86. The peptide of claim 79, wherein said one or more glycans is at least two glycans selected from an **N-linked** and an **O-linked** glycan.

87. The peptide of claim 79, wherein said peptide is expressed in a cell

selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

88. The peptide of claim 87, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell and a yeast cell.

L13 ANSWER 4 OF 12 USPATFULL on STN
2004:172476 Glycopegylation methods and proteins/peptides produced by the methods.
DeFrees, Shawn, North Wales, PA, UNITED STATES
Zopf, David, Wayne, PA, UNITED STATES
Bayer, Robert, San Diego, CA, UNITED STATES
Bowe, Caryn, Doylestown, PA, UNITED STATES
Hakes, David, Willow Grove, PA, UNITED STATES
Chen, Xi, Lansdale, PA, UNITED STATES
Neose Technologies, Inc. (U.S. corporation)
US 2004132640 A1 20040708
APPLICATION: US 2003-411012 A1 20030409 (10)
PRIORITY: US 2002-407527P 20020828 (60)
US 2002-404249P 20020816 (60)
US 2002-396594P 20020717 (60)
US 2002-391777P 20020625 (60)
US 2002-387292P 20020607 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A cell-free, in vitro method of remodeling a peptide comprising poly(ethylene glycol), said peptide having the formula: ##STR144## wherein AA is a terminal or internal amino acid residue of said peptide; X1-X2 is a saccharide covalently linked to said AA, wherein X1 is a first glycosyl residue; and X2 is a second glycosyl residue covalently linked to X1, wherein X1 and X2 are selected from monosaccharyl and oligosaccharyl residues; said method comprising: (a) removing X2 or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor comprising poly(ethylene glycol) under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby making a peptide comprising poly(ethylene glycol).

2. The method of claim 1, further comprising: (c) removing X1, thereby exposing said AA; and (d) contacting said AA with at least one glycosyltransferase and at least one glycosyl donor comprising poly(ethylene glycol) under conditions suitable to transfer said at least one glycosyl donor to said AA, thereby making said peptide comprising poly(ethylene glycol).

3. The method of claim 1, further comprising: (e) prior to step (b), removing a group added to said saccharide during post-translational modification.

4. The method of claim 3, wherein said group is a member selected from phosphate, sulfate, carboxylate and esters thereof.

5. The method of claim 1, wherein said peptide has the formula: ##STR145## wherein Z is a member selected from O, S, NH and a cross-linker.

6. The method of claim 1, wherein said poly(ethylene glycol) has a

molecular weight distribution that is essentially homodisperse.

7. A cell-free in vitro method of making a peptide comprising poly(ethylene glycol), said peptide having the formula: ##STR146## wherein X_3, X_4, X_5, X_6, X_7, and X_{17}, are independently selected monosaccharyl or oligosaccharyl residues; and a, b, c, d, e, and x are independently selected from the integers 0, 1 and 2, with the proviso that at least one member selected from a, b, c, d, e and x is 1 or 2; said method comprising: (a) removing at least one of X_3, X_4, X_5, X_6, X_7, X_{17}, or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor comprising poly(ethylene glycol) under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby making a peptide comprising poly(ethylene glycol).

8. The method of claim 7, wherein said removing of step (a) produces a truncated glycan in which a, b, c, e and x are each 0.

9. The method of claim 7, wherein X_3 X_5 and X_7 are selected from the group consisting of (mannose), and (mannose)$_z$-$(X_8)_y$ wherein X_8 is a glycosyl moiety selected from mono- and oligo-saccharides; y is an integer selected from 0 and 1; and z is an integer between 1 and 20, wherein when z is 3 or greater, (mannose), is selected from linear and branched structures.

10. The method of claim 7, wherein X_4 is selected from the group consisting of GlcNAc and xylose.

11. The method of claim 7, wherein X_3 X_5 and X_7 are (mannose)$_u$, wherein u is selected from the integers between 1 and 20, and when u is 3 or greater, (mannose)$_u$ is selected from linear and branched structures.

12. The method of claim 7, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

13. A cell-free in vitro method of remodeling a peptide comprising poly(ethylene glycol), said peptide comprising a glycan having the formula: ##STR147## wherein r, s, and t are integers independently selected from 0 and 1, said method comprising: (a) contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said glycan, thereby remodeling said peptide.

14. The method of claim 13, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

15. The method of claim 1, wherein said peptide has the formula: ##STR148## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1.

16. The method of claim 1, wherein said peptide has the formula: ##STR149## wherein X_{11} and X_{12} are independently selected glycosyl moieties; and r and x are integers independently selected from 0 and 1.

17. The method of claim 16, wherein X_{11} and X_{12} are (mannose)$_q$, wherein q is selected from the integers between 1 and

20, and when q is three or greater, $(\text{mannose})_q$ is selected from linear and branched structures.

18. The method of claim 1, wherein said peptide has the formula: ##STR150## wherein X_{13}, X_{14}, and X_{15} are independently selected glycosyl residues; and g, h, i, j, k, and p are independently selected from the integers 0 and 1, with the proviso that at least one of g, h, i, j, k and p is 1.

19. The method of claim 18, wherein X_{14} and X_{15} are members independently selected from GlcNAc and Sia; and i and k are independently selected from the integers 0 and 1, with the proviso that at least one of i and k is 1, and if k is 1, g, h, and j are 0.

20. The method of claim 1, wherein said peptide has the formula: ##STR151## wherein X_{16} is a member selected from: ##STR152## wherein s and i are integers independently selected from 0 and 1.

21. The method of claim 1, wherein said removing utilizes a glycosidase.

22. A cell-free, in vitro method of remodeling a peptide comprising poly(ethylene glycol), said peptide having the formula: ##STR153## wherein AA is a terminal or internal amino acid residue of said peptide; X_1 is a glycosyl residue covalently linked to said AA, selected from monosaccharyl and oligosaccharyl residues; and u is an integer selected from 0 and 1, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, wherein said glycosyl donor comprises a modifying group, thereby remodeling said peptide.

23. The method of claim 22, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

24. A covalent conjugate between a peptide and a poly(ethylene glycol) molecule, wherein said poly(ethylene glycol) molecule is covalently attached to said peptide at a preselected glycosyl or amino acid residue of said peptide via an intact glycosyl linking group.

25. The covalent conjugate of claim 24, wherein said poly(ethylene glycol) molecule and an intact glycosyl linking group precursor are linked as a covalently attached unit to said peptide via the action of an enzyme, said enzyme converting said precursor to said intact glycosyl linking group, thereby forming said conjugate.

26. The covalent conjugate of claim 25 comprising: a first modifying group covalently linked to a first residue of said peptide via a first intact glycosyl linking group, and a second glycosyl linking group linked to a second residue of said peptide via a second intact glycosyl linking group.

27. The covalent conjugate of claim 26, wherein said first residue and said second residue are structurally identical.

28. The covalent conjugate of claim 26, wherein said first residue and said second residue have different structures.

29. The covalent conjugate of claim 26 wherein said first residue and said second residue are glycosyl residues.

30. The covalent conjugate of claim 26, wherein said first residue and said second residue are amino acid residues.

31. The covalent conjugate of claim 26, wherein said peptide is remodeled prior to forming said conjugate.

32. The covalent conjugate of claim 31, wherein the remodeled peptide is remodeled to introduce an acceptor moiety for said intact glycosyl linking group.

33. The covalent conjugate of claim 26, wherein said intact glycosyl linking unit is a member selected from the group consisting of a sialic acid residue, a Gal residue, a GlcNAc residue and a GalNAc residue.

34. The covalent conjugate of claim 26, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

35. A method of forming a covalent conjugate between a poly(ethylene glycol) molecule and a **glycosylated** or non-**glycosylated** peptide, wherein said poly(ethylene glycol) molecule is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said poly(ethylene glycol) molecule, said method comprising: contacting said peptide with a mixture comprising a nucleotide sugar covalently linked to said poly(ethylene glycol) molecule and a glycosyltransferase for which said nucleotide sugar is a substrate under conditions sufficient to form said conjugate.

36. The method of claim 35, wherein said glycosyl linking group is covalently attached to a glycosyl residue covalently attached to said peptide.

37. The method of claim 35, wherein said glycosyl linking group is covalently attached to an amino acid residue of said peptide.

38. The method of claim 35, wherein said poly(ethylene glycol) has a degree of polymerization of from about 1 to about 20,000.

39. The method of claim 35, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 5,000.

40. The method of claim 35, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 1,000.

41. The method of claim 35, wherein said glycosyltransferase is selected from the group consisting of sialyltransferase, galactosyltransferase, glucosyltransferase, GalNAc transferase, GlcNAc transferase, fucosyltransferase, and mannosyltransferase.

42. The method of claim 35, wherein said glycosyltransferase is recombinantly produced.

43. The method of claim 42, wherein said glycosyltransferase is a recombinant prokaryotic enzyme.

44. The method of claim 42, wherein said glycosyltransferase is a recombinant eukaryotic enzyme.

45. The method of claim 35, wherein said nucleotide sugar is selected from the group consisting of UDP-glycoside, CMP-glycoside, and GDP-glycoside.

46. The method of claim 45, wherein said nucleotide sugar is selected

from the group consisting of UDP-galactose, UDP-galactosamine, UDP-glucose, UDP-glucosamine, UDP-N-acetylgalactosamine, UDP-N-acetylglucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid, CMP-NeuAc.

47. The method of claim 35, wherein said peptide is a therapeutic agent.

48. A method of claim 35, wherein said **glycosylated** peptide is partially deglycosylated prior to said contacting.

49. A method of claim 35, wherein said intact glycosyl linking group is a sialic acid residue.

50. The method of claim 35, wherein said method is performed in a cell-free **environment**.

51. The method of claim 35, wherein said covalent conjugate is isolated.

52. The method of claim 51, wherein said covalent conjugate is isolated by membrane filtration.

53. A method of forming a covalent conjugate between and a first **glycosylated** or non-**glycosylated** peptide, and a second **glycosylated** or non-**glycosylated** peptide cojoined by a poly(ethylene glycol) molecule, wherein said poly(ethylene glycol) molecule is conjugated to said first peptide via a first intact glycosyl linking group interposed between and covalently linked to both said first peptide and said poly(ethylene glycol) molecule, and said poly(ethylene glycol) molecule is conjugated to said second peptide via a second intact glycosyl linking group interposed between and covalently linked to both said second peptide and said poly(ethylene glycol) molecule; said method comprising: (a) contacting said first peptide with a derivative of said poly(ethylene glycol) molecule precursor comprising a precursor of said first intact glycosyl linking group and a precursor of said second intact glycosyl linking group; (b) contacting the mixture from (a) with a glycosyl transferase for which said precursor of said first glycosyl linking group is a substrate, under conditions sufficient to convert said precursor of said first intact glycosyl linking group into said first intact glycosyl linking group, thereby forming a first conjugate between said poly(ethylene glycol) molecule precursor and said first peptide; (c) contacting said first conjugate with said second peptide and a glycosyltransferase for which said precursor of said second intact glycosyl group is a substrate under conditions sufficient to convert said precursor of said second intact glycosyl linking group into said second glycosyl linking group, thereby forming said conjugate between said poly(ethylene glycol) molecule and said first **glycosylated** or non-**glycosylated** peptide, and said second **glycosylated** or non-**glycosylated** peptide.

54. A method of forming a covalent conjugate between and a first **glycosylated** or non-**glycosylated** peptide, and a second **glycosylated** or non-**glycosylated** peptide cojoined by a poly(ethylene glycol) molecule, wherein said poly(ethylene glycol) molecule is covalently conjugated to said first peptide, and said poly(ethylene glycol) molecule is conjugated to said second peptide via an intact glycosyl linking group interposed between and covalently linked to both said second peptide and poly(ethylene glycol) molecule, said method comprising: (a) contacting said first peptide with an activated derivative of said poly(ethylene glycol) molecule comprising; a reactive functional group of reactivity complementary to a residue on said first peptide, and a precursor of said intact glycosyl linking group, under conditions sufficient to form a covalent bond between said

reactive functional group and said residue, thereby forming a first conjugate; and (b) contacting said first conjugate with said second peptide and a glycosyltransferase for which said precursor of said intact glycosyl linking group is a substrate, under conditions sufficient to convert said precursor of said intact glycosyl linking group into said intact glycosyl linking group, thereby forming said conjugate between said first **glycosylated** or non-**glycosylated** peptide, and said second **glycosylated** or non-**glycosylated** peptide cojoined by said poly(ethylene glycol) molecule.

55. A pharmaceutical composition comprising a pharmaceutically acceptable diluent and a covalent conjugate between a poly(ethylene glycol) molecule and a **glycosylated** or non-**glycosylated** peptide, wherein said poly(ethylene glycol) molecule is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said poly(ethylene glycol) molecule.

56. A composition for forming a conjugate between a peptide and a modified sugar, said composition comprising: an admixture of a modified sugar, a glycosyltransferase, and a peptide acceptor substrate, wherein said modified sugar has covalently attached thereto a poly(ethylene glycol) molecule.

57. A peptide comprising poly(ethylene glycol) made by the method of claim 1.

58. A pharmaceutical composition comprising the peptide of claim 57.

59. A peptide comprising poly(ethylene glycol) made by the method of claim 7.

60. A pharmaceutical composition comprising the peptide of claim 59.

61. A peptide comprising poly(ethylene glycol) made by the method of claim 13.

62. A pharmaceutical composition comprising the peptide of claim 61.

63. A peptide comprising poly(ethylene glycol) made by the method of claim 22.

64. A pharmaceutical composition comprising the peptide of claim 63.

65. A peptide comprising poly(ethylene glycol) made by the method of claim 15.

66. A pharmaceutical composition comprising the peptide of claim 65.

67. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR154## wherein AA is a terminal or internal amino acid residue of said peptide, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said amino acid residue, wherein said glycosyl donor comprises poly (ethylene glycol), thereby remodeling said peptide.

68. A peptide comprising one or more glycans, having a poly(ethylene glycol) molecule covalently attached to said peptide, wherein said poly(ethylene glycol) molecule is added to said one or more glycans using a glycosyltransferase.

69. The peptide of claim 68, wherein said one or more glycans is a monoantennary glycan.

70. The peptide of claim 68, wherein said one or more glycans is a biantennary glycan.

71. The peptide of claim 70, wherein said one or more glycans is a triantennary glycan.

72. The peptide of claim 71, wherein said one or more glycans is at least a triantennary glycan.

73. The peptide of claim 71, wherein said one or more glycans comprises at least two glycans comprising a mixture of mono and multiantennary glycans.

74. The peptide of claim 71, wherein said one or more glycans is selected from an **N-linked** glycan and an **O-linked** glycan.

75. The peptide of claim 71, wherein said one or more glycans is at least two glycans selected from an **N-linked** and an **O-linked** glycan.

76. The peptide of claim 71, wherein said peptide is expressed in a cell selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

77. The peptide of claim 76, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell and a yeast cell.

L13 ANSWER 5 OF 12 USPATFULL on STN
2004:165351 Follicle stimulating hormone: remodeling and glycoconjugation of FSH.
DeFrees, Shawn, North Wales, PA, UNITED STATES
Zopf, David, Wayne, PA, UNITED STATES
Bayer, Robert, San Diego, CA, UNITED STATES
Bowe, Caryn, Doylestown, PA, UNITED STATES
Hakes, David, Willow Grove, PA, UNITED STATES
Chen, Xi, Lansdale, PA, UNITED STATES
Neose Technologies, Inc. (U.S. corporation)
US 2004126838 A1 20040701
APPLICATION: US 2003-410997 A1 20030409 (10)
PRIORITY: US 2002-407527P 20020828 (60)
US 2002-404249P 20020816 (60)
US 2002-396594P 20020717 (60)
US 2002-391777P 20020625 (60)
US 2002-387292P 20020607 (60)
US 2001-334301P 20011128 (60)
US 2001-334233P 20011128 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A cell-free, in vitro method of remodeling a follicle stimulating hormone (FSH) peptide, said peptide having the formula: ##STR148## wherein AA is a terminal or internal amino acid residue of said peptide; X_1-X_2 is a saccharide covalently linked to said AA, wherein X_1 is a first glycosyl residue; and X_2 is a second glycosyl residue covalently linked to X_1, wherein X_1 and X_2 are selected from monosaccharyl and oligosaccharyl residues; said method comprising: (a) removing X_2 or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b)

contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said FSH peptide.

2. The method of claim 1, further comprising: (c) removing X_1, thereby exposing said AA; and (d) contacting said AA with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said AA, thereby remodeling said FSH peptide.

3. The method of claim 1, further comprising: (e) prior to step (b), removing a group added to said saccharide during post-translational modification.

4. The method of claim 3, wherein said group is a member selected from phosphate, sulfate, carboxylate and esters thereof.

5. The method of claim 1, wherein said peptide has the formula: ##STR149## wherein Z is a member selected from O, S, NH and a crosslinker.

6. The method of claim 1, wherein at least one of said glycosyl donors comprises a modifying group.

7. The method of claim 1, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

8. The method of claim 7, wherein said modifying group is a water soluble polymer.

9. The method of claim 8, wherein said water soluble polymer comprises poly(ethylene glycol).

10. The method of claim 9, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

11. A cell-free in vitro method of remodeling a FSH peptide, said peptide having the formula: ##STR150## wherein X_3, X_4, X_5, X_6, X_7, and X_{17} are independently selected monosaccharyl or oligosaccharyl residues; and a, b, c, d, e and x are independently selected from the integers 0, 1 and 2, with the proviso that at least one member selected from a, b, c, d, and e and x are 1 or 2; said method comprising: (a) removing at least one of X_3, X_4, X_5, X_6, X_7, or X_{17}, a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said FSH peptide.

12. The method of claim 11, wherein said removing of step (a) produces a truncated glycan in which a, b, c, e and x are each 0.

13. The method of claim 11, wherein X_3, X_5, and X_7, are selected from the group consisting of (mannose), and (mannose)$_z$-$(X_8)_y$ wherein X_8 is a glycosyl moiety selected from mono- and oligo-saccharides; y is an integer selected from 0 and 1; and z is an integer between 1 and 20, wherein when z is 3 or greater, (mannose), is selected from linear and branched

structures.

14. The method of claim 11, wherein X_4 is selected from the group consisting of GlcNAc and xylose.

15. The method of claim 11, wherein X_3, X_5, and X_7 are (mannose)u, wherein u is selected from the integers between 1 and 20, and when u is 3 or greater, (mannose)u is selected from linear and branched structures.

16. The method of claim 11, wherein at least one of said glycosyl donors comprises a modifying group.

17. The method of claim 16, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

18. The method of claim 17 wherein said modifying group is a water soluble polymer.

19. The method of claim 18, wherein said water soluble polymer comprises poly(ethylene glycol).

20. The method of claim 19, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

21. A cell-free in vitro method of remodeling a FSH peptide comprising a glycan having the formula: ##STR151## wherein r, s, and t are integers independently selected from 0 and 1, said method comprising: (a) contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said glycan, thereby remodeling said FSH peptide.

22. The method of claim 21, wherein at least one of said glycosyl donors comprises a modifying group.

23. The method of claim 22, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

24. The method of claim 23 wherein said modifying group is a water soluble polymer.

25. The method of claim 24, wherein said water soluble polymer comprises poly(ethylene glycol).

26. The method of claim 25, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

27. The method of claim 1, wherein said peptide has the formula: ##STR152## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1.

28. The method of claim 1, wherein said peptide has the formula: ##STR153## wherein X_{11} and X_{12} are independently selected glycosyl moieties; and r and x are integers independently selected from 0 and 1.

29. The method of claim 28, wherein X_{11} and X_{12} are $(mannose)_q$, wherein q is selected from the integers between 1 and 20, and when q is three or greater, $(mannose)_q$ is selected from linear and branched structures.

30. The method of claim 1, wherein said peptide has the formula: ##STR154## wherein X_{13}, X_{14}, and X_{15} are independently selected glycosyl residues; and g, h, i,j, k, and p are independently selected from the integers 0 and 1, with the proviso that at least one of g, h, i, j, k and p is 1.

31. The method of claim 30, wherein X_{14} and X_{15} are members independently selected from GlcNAc and Sia; and i and k are independently selected from the integers 0 and 1, with the proviso that at least one of i and k is 1 and if k is 1, g, h and j are 0.

32. The method of claim 1, wherein said peptide has the formula: ##STR155## wherein X_{16} is a member selected from: ##STR156## wherein s and i are integers independently selected from 0 and 1.

33. The method of claim 1, wherein said removing utilizes a glycosidase.

34. A cell-free, in vitro method of remodeling a FSH peptide having the formula: ##STR157## wherein AA is a terminal or internal amino acid residue of said peptide; X_1 is a glycosyl residue covalently linked to said AA, selected from monosaccharyl and oligosaccharyl residues; and u is an integer selected from 0 and 1, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, wherein said glycosyl donor comprises a modifying group, thereby remodeling said FSH peptide.

35. The method of claim 34, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

36. The method of claim 35 wherein said modifying group is a water soluble polymer.

37. The method of claim 36, wherein said water soluble polymer comprises poly(ethylene glycol).

38. The method of claim 37, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

39. A covalent conjugate between a FSH peptide and a modifying group that alters a property of said peptide, wherein said modifying group is covalently attached to said peptide at a preselected glycosyl or amino acid residue of said peptide via an intact glycosyl linking group.

40. The covalent conjugate of claim 39, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

41. The covalent conjugate of claim 39, wherein said modifying group and an intact glycosyl linking group precursor are linked as a covalently attached unit to said peptide via the action of an enzyme, said enzyme converting said precursor to said intact glycosyl linking group, thereby forming said conjugate.

42. The covalent conjugate of claim 39 comprising: a first modifying group covalently linked to a first residue of said peptide via a first intact glycosyl linking group, and a second glycosyl linking group linked to a second residue of said peptide via a second intact glycosyl linking group.

43. The covalent conjugate of claim 42, wherein said first residue and said second residue are structurally identical.

44. The covalent conjugate of claim 42, wherein said first residue and said second residue have different structures.

45. The covalent conjugate of claim 42 wherein said first residue and said second residue are glycosyl residues.

46. The covalent conjugate of claim 42, wherein said first residue and said second residue are amino acid residues.

47. The covalent conjugate of claim 39, wherein said peptide is remodeled prior to forming said conjugate.

48. The covalent conjugate of claim 47, wherein the remodeled peptide is remodeled to introduce an acceptor moiety for said intact glycosyl linking group.

49. The covalent conjugate of claim 39, wherein said modifying group is a water-soluble polymer.

50. The covalent conjugate of claim 49, wherein said water-soluble polymer comprises poly(ethylene glycol).

51. The covalent conjugate of claim 39, wherein said intact glycosyl linking unit is a member selected from the group consisting of a sialic acid residue, a Gal residue, a GlcNAc residue, and a GalNAc residue.

52. The covalent conjugate of claim 50, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

53. A method of forming a covalent conjugate between a polymer and a **glycosylated** or non-**glycosylated** peptide, wherein said polymer is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said polymer, said method comprising: contacting said peptide with a mixture comprising a nucleotide sugar covalently linked to said polymer and a glycosyltransferase for which said nucleotide sugar is a substrate under conditions sufficient to form said conjugate, wherein said peptide is FSH.

54. The method of claim 53, wherein said polymer is a water-soluble polymer.

55. The method of claim 53, wherein said glycosyl linking group is covalently attached to a glycosyl residue covalently attached to said peptide.

56. The method of claim 53, wherein said glycosyl linking group is covalently attached to an amino acid residue of said peptide.

57. The method of claim 53, wherein said polymer comprises a member selected from the group consisting of a polyalkylene oxide and a

polypeptide.

58. The method of claim 57, wherein said polyalkylene oxide is poly(ethylene glycol).

59. The method of claim 58, wherein said poly(ethylene glycol) has a degree of polymerization of from about 1 to about 20,000.

60. The method of claim 59, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 5,000.

61. The method of claim 60, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 1,000.

62. The method of claim 53, wherein said glycosyltransferase is selected from the group consisting of sialyltransferase, galactosyltransferase, glucosyltransferase, GalNAc transferase, and a GlcNAc transferase.

63. The method of claim 53, wherein said glycosyltransferase is recombinantly produced.

64. The method of claim 63, wherein said glycosyltransferase is a recombinant prokaryotic enzyme.

65. The method of claim 63, wherein said glycosyltransferase is a recombinant eukaryotic enzyme.

66. The method of claim 53, wherein said nucleotide sugar is selected from the group consisting of UDP-glycoside, CMP-glycoside, and GDP-glycoside.

67. The method of claim 66, wherein said nucleotide sugar is selected from the group consisting of UDP-galactose, UDP-galactosamine, UDP-glucose, UDP-glucosamine, UDP-N-acetylgalactosamine, UDP-N-acetylglucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid, CMP-NeuAc.

68. The method of claim 53, wherein said **glycosylated** peptide is partially deglycosylated prior to said contacting.

69. The method of claim 53, wherein said intact glycosyl linking group is a sialic acid residue.

70. The method of claim 53, wherein said method is performed in a cell-free **environment**.

71. The method of claim 53, wherein said covalent conjugate is isolated.

72. The method of claim 71, wherein said covalent conjugate is isolated by membrane filtration.

73. A composition for forming a conjugate between a peptide and a modified sugar, said composition comprising: an admixture of a modified sugar, a glycosyltransferase, and a peptide acceptor substrate, wherein said modified sugar has covalently attached thereto a member selected from a polymer, a therapeutic moiety and a biomolecule, wherein said peptide is FSH.

74. A FSH peptide remodeled by the method of claim 1.

75. A pharmaceutical composition comprising the FSH peptide of claim 74.

76. A FSH peptide remodeled by the method of claim 11.

77. A pharmaceutical composition comprising the FSH peptide of claim 76.

78. A FSH peptide remodeled by the method of claim 21.

79. A pharmaceutical composition comprising the FSH peptide of claim 78.

80. A FSH peptide remodeled by the method of claim 27.

81. A pharmaceutical composition comprising the FSH peptide of claim 80.

82. A FSH peptide remodeled by the method of claim 28.

83. A pharmaceutical composition comprising the FSH peptide of claim 82.

84. A FSH peptide remodeled by the method of claim 34.

85. A pharmaceutical composition comprising the FSH peptide of claim 84.

86. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR158## wherein AA is a terminal or internal amino acid residue of said peptide, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said amino acid residue, wherein said glycosyl donor comprises a modifying group, thereby remodeling said peptide, wherein said peptide is FSH.

87. A method of forming a conjugate between a FSH peptide and a modifying group, wherein said modifying group is covalently attached to said FSH peptide through an intact glycosyl linking group, said FSH peptide comprising a glycosyl residue having the formula: ##STR159## wherein a, b, c, d, i, q, r, s, t, and u are members independently selected from 0 and 1; e, f, g, and h are members independently selected from the integers between 0 and 6; j, k, l, and m are members independently selected from the integers between 0 and 100; v, w, x, and y are 0; and R is a modifying group, a mannose or an oligomannose; said method comprising: (a) contacting said FSH peptide with a glycosyltransferase and a modified glycosyl donor, comprising a glycosyl moiety which is a substrate for said glycosyltransferase covalently linked to said modifying group, under conditions appropriate for the formation of said intact glycosyl linking group.

88. The method of claim 87, further comprising: (b) prior to step (a), contacting said FSH peptide with a sialidase under conditions appropriate to remove sialic acid from said FSH peptide.

89. The method of claim 87, further comprising: (c) contacting the product of step (a) with a sialyltransferase and a sialic acid donor under conditions appropriate to transfer sialic acid to said product.

90. The method of claim 87, further comprising: (d) prior to step (a), contacting said FSH peptide with a galactosidase under conditions appropriate to remove galactose from said FSH peptide.

91. The method of claim 87, further comrprising: (e) prior to step (a) contacting said FSH peptide with a combination of a glycosidase and a sialidase.

92. The method of claim 87, further comprising: (f) prior to step (a), contacting said FSH peptide with a galactosyl transferase and a

galactose donor under conditions appropriate to transfer said galactose to said FSH peptide.

93. The method of claim 87, further comprising: (d) contacting the product from step (a) with a moiety that reacts with said modifying group, thereby forming a conjugate between said intact glycosyl linking group and said moiety.

94. The method of claim 87, further comprising: (e) prior to step (b), contacting said FSH peptide with an endoglycanase under conditions appropriate to cleave a glycosyl moiety from said FSH peptide.

95. The method of claim 87, further comprising: (f) prior to step (a), contacting said FSH peptide with N-acetylglucosarine transferase and a GlcNAc donor under conditions appropriate to transfer GlcNAc to said FSH peptide.

96. The method of claim 87, wherein said modifying group is a member selected from a polymer, a toxin, a radioisotope, a therapeutic moiety and a glycoconjugate.

97. The method of claim 87, wherein a, b, c, d, i, j, k, l, m, q, r, s, t, and u are members independently selected from 0 and 1; e, f, g, and hare 1; and v, w, x, and y are 0.

98. The method of claim 87, wherein a, b, c, d, e, f, g, h, i, j, k, l, m, q, r, s, t, and u are members independently selected from 0 and 1; v, w, x, and y are 0.

99. The method of claim 87, wherein a, b, c, d, f, h, j, k, l, m , s, u, v, w, x, and y are 0; and e, g, i, q, r, and t are members independently selected from 0 and 1.

100. The method of claim 87, wherein a, b, c, d, e, f, g, h, j, k, l, and m are 0; i, q, r, s, t, u, v, w, x, and y are independently selected from 0 and 1; p is 1; R (branched or linear) is a member selected from mannose and oligomannose.

101. The method of claim 87, wherein a, b, c, d, e, f, g, h, j, k, l, m, r, s, t, u, v, w, and y are 0; i is 0 or 1; and q is 1.

102. A FSH peptide conjugate formed by the method of claim 87.

103. A FSH peptide comprising one or more glycans, having a glycoconjugate molecule covalently attached to said peptide.

104. The FSH peptide of claim 103, wherein said one or more glycans is a monoantennary glycan.

105. The FSH peptide of claim 103, wherein said one or more glycans is a biantennary glycan.

106. The FSH peptide of claim 103, wherein said one or more glycans is a triantennary glycan.

107. The FSH peptide of claim 103, wherein said one or more glycans is at least a triantennary glycan.

108. The FSH peptide of claim 103, wherein said one or more glycans comprises at least two glycans comprising a mixture of mono or multiantennary glycans.

109. The FSH peptide of claim 103, wherein said one or more glycans is selected from an **N-linked** glycan and an **O-linked** glycan.

110. The FSH peptide of claim 103, wherein said one or more glycans is at least two glycans selected from an **N-linked** and an **O-linked** glycan.

111. The FSH peptide of claim 103, wherein said peptide is expressed in a cell selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

112. The FSH peptide of claim 111, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell and a yeast cell.

113. A method of stimulating follicular maturation in a female mammal, said method comprising administering to said mammal a FSH peptide having one or more glycans having a glycoconjugate molecule attached to said peptide.

114. The method of claim 113, wherein said glycoconjugate is poly(ethylene glycol).

115. The method of claim 114, wherein said mammal is a human.

L13 ANSWER 6 OF 12 USPATFULL on STN
2004:150947 Interferon beta: remodeling and glycoconjugation of interferon beta
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DeFrees, Shawn, North Wales, PA, UNITED STATES
Zopf, David, Wayne, PA, UNITED STATES
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Neose Technologies, Inc. (U.S. corporation)
US 2004115168 A1 20040617
APPLICATION: US 2003-410930 A1 20030409 (10)
PRIORITY: US 2002-407527P 20020828 (60)
US 2002-404249P 20020816 (60)
US 2002-396594P 20020717 (60)
US 2002-391777P 20020625 (60)
US 2002-387292P 20020607 (60)
US 2001-334301P 20011128 (60)
US 2001-334233P 20011128 (60)
US 2001-344692P 20011019 (60)
US 2001-328523P 20011010 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A cell-free, in vitro method of remodeling an interferon beta peptide, said peptide having the formula: ##STR150## wherein AA is a terminal or internal amino acid residue of said peptide; X_1-X_2 is a saccharide covalently linked to said AA, wherein X_1 is a first glycosyl residue; and X_2 is a second glycosyl residue covalently linked to X_1, wherein X_1 and X_2 are selected from monosaccharyl and oligosaccharyl residues; said method comprising: (a) removing X_2 or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said interferon beta peptide.

2. The method of claim 1, further comprising: (c) removing X_1, thereby exposing said AA; and (d) contacting said AA with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said AA, thereby remodeling said interferon beta peptide.

3. The method of claim 1, further comprising: (e) prior to step (b), removing a group added to said saccharide during post-translational modification.

4. The method of claim 3, wherein said group is a member selected from phosphate, sulfate, carboxylate and esters thereof.

5. The method of claim 1, wherein said peptide has the formula: ##STR151## wherein Z is a member selected from O, S, NH and a crosslinker.

6. The method of claim 1, wherein at least one of said glycosyl donors comprises a modifying group.

7. The method of claim 1, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

8. The method of claim 7, wherein said modifying group is a water soluble polymer.

9. The method of claim 8, wherein said water soluble polymer comprises poly(ethylene glycol).

10. The method of claim 9, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

11. A cell-free in vitro method of remodeling an interferon beta peptide, said peptide having the formula: ##STR152## wherein X_3, X_4, X_5, X_6, X_7, and X_{17} are independently selected monosaccharyl or oligosaccharyl residues; and a, b, c, d, e and x are independently selected from the integers 0, 1 and 2, with the proviso that at least one member selected from a, b, c, d, and e and x are 1 or 2; said method comprising: (a) removing at least one of X_3, X_4, X_5, X_6, X_7, or X_{17}, a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said interferon beta peptide.

12. The method of claim 11, wherein said removing of step (a) produces a truncated glycan in which a, b, c, e and x are each 0.

13. The method of claim 11, wherein X_3, X_5, and X_7, are selected from the group consisting of $(\text{mannose})_z$ and $(\text{mannose})_z\text{-}(X_8)_y$ wherein X_8 is a glycosyl moiety selected from mono- and oligo-saccharides; y is an integer selected from 0 and 1; and z is an integer between 1 and 20, wherein when z is 3 or greater, $(\text{mannose})_z$ is selected from linear and branched structures.

14. The method of claim 11, wherein X_4 is selected from the group consisting of GlcNAc and xylose.

15. The method of claim 11, wherein X_3, X_5, and X_7 are (mannose)u, wherein u is selected from the integers between 1 and 20, and when u is 3 or greater, (mannose)u is selected from linear and branched structures.

16. The method of claim 11, wherein at least one of said glycosyl donors comprises a modifying group.

17. The method of claim 16, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

18. The method of claim 17 wherein said modifying group is a water soluble polymer.

19. The method of claim 18, wherein said water soluble polymer comprises poly(ethylene glycol).

20. The method of claim 19, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

21. A cell-free in vitro method of remodeling an interferon beta peptide comprising a glycan having the formula: ##STR153## wherein r, s, and t are integers independently selected from 0 and 1, said method comprising: (a) contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said glycan, thereby remodeling said interferon beta peptide.

22. The method of claim 21, wherein at least one of said glycosyl donors comprises a modifying group.

23. The method of claim 22, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

24. The method of claim 23 wherein said modifying group is a water soluble polymer.

25. The method of claim 24, wherein said water soluble polymer comprises poly(ethylene glycol).

26. The method of claim 25, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

27. The method of claim 1, wherein said peptide has the formula: ##STR154## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1.

28. The method of claim 1, wherein said peptide has the formula: ##STR155## wherein X_{11} and X_{12} are independently selected glycosyl moieties; and r and x are integers independently selected from 0 and 1.

29. The method of claim 28, wherein X_{11} and X_{12} are (mannose)q, wherein q is selected from the integers between 1 and 20, and when q is three or greater, (mannose)q is selected from linear and branched structures.

30. The method of claim 1, wherein said peptide has the formula: ##STR156## wherein X_{13}, X_{14}, and X_{15} are independently selected glycosyl residues; and g, h, i, j, k, and p are independently selected from the integers 0 and 1, with the proviso that at least one of g, h, i, j, k and p is 1.

31. The method of claim 30, wherein X_{14} and X_{15} are members independently selected from GlcNAc and Sia; and i and k are independently selected from the integers 0 and 1, with the proviso that at least one of i and k is 1 and if k is 1, g, h and j are 0.

32. The method of claim 1, wherein said peptide has the formula: ##STR157## wherein X_{16} is a member selected from: ##STR158## wherein s and i are integers independently selected from 0 and 1.

33. The method of claim 1, wherein said removing utilizes a glycosidase.

34. A cell-free, in vitro method of remodeling an interferon beta peptide having the formula: ##STR159## wherein AA is a terminal or internal amino acid residue of said peptide; X_1 is a glycosyl residue covalently linked to said AA, selected from monosaccharyl and oligosaccharyl residues; and u is an integer selected from 0 and 1, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, wherein said glycosyl donor comprises a modifying group, thereby remodeling said interferon beta peptide.

35. The method of claim 34, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

36. The method of claim 35 wherein said modifying group is a water soluble polymer.

37. The method of claim 36, wherein said water soluble polymer comprises poly(ethylene glycol).

38. The method of claim 37, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

39. A covalent conjugate between a interferon beta peptide and a modifying group that alters a property of said peptide, wherein said modifying group is covalently attached to said peptide at a preselected glycosyl or amino acid residue of said peptide via an intact glycosyl linking group.

40. The covalent conjugate of claim 39, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

41. The covalent conjugate of claim 39, wherein said modifying group and an intact glycosyl linking group precursor are linked as a covalently attached unit to said peptide via the action of an enzyme, said enzyme converting said precursor to said intact glycosyl linking group, thereby forming said conjugate.

42. The covalent conjugate of claim 39 comprising: a first modifying group covalently linked to a first residue of said peptide via a first

intact glycosyl linking group, and a second glycosyl linking group linked to a second residue of said peptide via a second intact glycosyl linking group.

43. The covalent conjugate of claim 42, wherein said first residue and said second residue are structurally identical.

44. The covalent conjugate of claim 42, wherein said first residue and said second residue have different structures.

45. The covalent conjugate of claim 42 wherein said first residue and said second residue are glycosyl residues.

46. The covalent conjugate of claim 42, wherein said first residue and said second residue are amino acid residues.

47. The covalent conjugate of claim 39, wherein said peptide is remodeled prior to forming said conjugate.

48. The covalent conjugate of claim 47, wherein the remodeled peptide is remodeled to introduce an acceptor moiety for said intact glycosyl linking group.

49. The covalent conjugate of claim 39, wherein said modifying group is a water-soluble polymer.

50. The covalent conjugate of claim 49, wherein said water-soluble polymer comprises poly(ethylene glycol).

51. The covalent conjugate of claim 39, wherein said intact glycosyl linking unit is a member selected from the group consisting of a sialic acid residue, a Gal residue, a GlcNAc residue and a GalNAc residue.

52. The covalent conjugate of claim 50, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

53. A method of forming a covalent conjugate between a polymer and a **glycosylated** or non-**glycosylated** peptide, wherein said polymer is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said polymer, said method comprising: contacting said peptide with a mixture comprising a nucleotide sugar covalently linked to said polymer and a glycosyltransferase for which said nucleotide sugar is a substrate under conditions sufficient to form said conjugate, wherein said peptide is interferon beta.

54. The method of claim 53, wherein said polymer is a water-soluble polymer.

55. The method of claim 53, wherein said glycosyl linking group is covalently attached to a glycosyl residue covalently attached to said peptide.

56. The method of claim 53, wherein said glycosyl linking group is covalently attached to an amino acid residue of said peptide.

57. The method of claim 53, wherein said polymer comprises a member selected from the group consisting of a polyalkylene oxide and a polypeptide.

58. The method of claim 57, wherein said polyalkylene oxide is

poly(ethylene glycol).

59. The method of claim 58, wherein said poly(ethylene glycol) has a degree of polymerization of from about 1 to about 20,000.

60. The method of claim 59, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 5,000.

61. The method of claim 60, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 1,000.

62. The method of claim 53, wherein said glycosyltransferase is selected from the group consisting of sialyltransferase, galactosyltransferase, glucosyltransferase, GalNAc transferase, GlcNAc transferase, fucosyltransferase, and mannosyltransferase.

63. The method of claim 53, wherein said glycosyltransferase is recombinantly produced.

64. The method of claim 63, wherein said glycosyltransferase is a recombinant prokaryotic enzyme.

65. The method of claim 63, wherein said glycosyltransferase is a recombinant eukaryotic enzyme.

66. The method of claim 53, wherein said nucleotide sugar is selected from the group consisting of UDP-glycoside, CMP-glycoside, and GDP-glycoside.

67. The method of claim 66, wherein said nucleotide sugar is selected from the group consisting of UDP-galactose, UDP-galactosamine, UDP-glucose, UDP-glucosamine, UDP-N-acetylgalactosamine, UDP-N-acetylglucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid, CMP-NeuAc.

68. The method of claim 53, wherein said **glycosylated** peptide is partially deglycosylated prior to said contacting.

69. The method of claim 53, wherein said intact glycosyl linking group is a sialic acid residue.

70. The method of claim 53, wherein said method is performed in a cell-free **environment**.

71. The method of claim 53, wherein said covalent conjugate is isolated.

72. The method of claim 71, wherein said covalent conjugate is isolated by membrane filtration.

73. A composition for forming a conjugate between a peptide and a modified sugar, said composition comprising: an admixture of a modified sugar, a glycosyltransferase, and a peptide acceptor substrate, wherein said modified sugar has covalently attached thereto a member selected from a polymer, a therapeutic moiety and a biomolecule, wherein said peptide is interferon beta.

74. An interferon beta peptide remodeled by the method of claim 1.

75. A pharmaceutical composition comprising the interferon beta peptide of claim 74.

76. An interferon beta peptide remodeled by the method of claim 11.

77. A pharmaceutical composition comprising the interferon beta peptide of claim 76.

78. An interferon beta peptide remodeled by the method of claim 21.

79. A pharmaceutical composition comprising the interferon beta peptide of claim 78.

80. An interferon beta peptide remodeled by the method of claim 27.

81. A pharmaceutical composition comprising the interferon beta peptide of claim 80.

82. An interferon beta peptide remodeled by the method of claim 28.

83. A pharmaceutical composition comprising the interferon beta peptide of claim 82.

84. An interferon beta peptide remodeled by the method of claim 34.

85. A pharmaceutical composition comprising the interferon beta peptide of claim 82.

86. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR160## wherein AA is a terminal or internal amino acid residue of said peptide, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said amino acid residue, wherein said glycosyl donor comprises a modifying group, thereby remodeling said peptide, wherein said peptide is interferon beta.

87. A method of forming a conjugate between an interferon beta peptide and a modifying group, wherein said modifying group is covalently attached to said interferon beta peptide through an intact glycosyl linking group, said interferon beta peptide comprising a glycosyl residue having the formula: ##STR161## wherein a, b, c, d, i, p, q, r, s, t, and u are members independently selected from 0 and 1; e, f, g, and h are members independently selected from the integers between 0 and 6; j, k, l, and m are members independently selected from the integers between 0 and 100; v, w, x, and y are 0; R is a modifying group, mannose or oligomannose; and R' is H or a glycosyl, modifying group or glycoconjugate group, said method comprising: (a) contacting said interferon beta peptide with a member selected from a glycosyltransferase and a sialyltransferase and a modified glycosyl donor, comprising a glycosyl moiety which is a substrate for said glycosyltransferase covalently linked to said modifying group, under conditions appropriate for the formation of said intact glycosyl linking group.

88. The method of claim 87, further comprising: (b) prior to step (a), contacting said interferon beta peptide with a sialidase under conditions appropriate to remove sialic acid from said interferon beta peptide.

89. The method of claim 87, further comprising: (c) contacting the product from step (a) with a moiety that reacts with said modifying group, thereby forming a conjugate between said intact glycosyl linking group and said moiety.

90. The method of claim 87, further comprising: (d) prior to step (a)

contacting said interferon beta peptide with a combination of a glycosidase and a sialidase.

91. The method of claim 87, further comprising: (e) prior to step (a), contacting said interferon beta peptide with an endoglycanase under conditions appropriate to cleave a glycosyl moiety from said interferon beta peptide.

92. The method of claim 87, further comprising: (f) prior to step (a), contacting said interferon beta peptide with N-acetylglucosamine transferase and a GlcNAc donor under conditions appropriate to transfer GlcNAc to said interferon beta peptide.

93. The method of claim 87, further comprising: (g) prior to step (a), contacting said interferon beta peptide with a galactosyl transferase and a galactose donor under conditions appropriate to transfer galactose to said product.

94. The method of claim 87, further comprising: (h) prior to step (b), contacting said interferon beta peptide with endoglycanase under conditions appropriate to cleave a glycosyl moiety from said interferon beta peptide.

95. The method of claim 87, further comprising: (i) prior to step (a), contacting said interferon beta peptide with a mannosidase under conditions appropriate to remove mannose from said interferon beta peptide.

96. The method of claim 87, further comprising: (j) contacting the product of step (a) with a sialyltransferase and a sialic acid donor under conditions appropriate to transfer sialic acid to said product.

97. The method of claim 87, wherein said modifying group is a member selected from a polymer, a toxin, a radioisotope, a therapeutic moiety and a glycoconjugate.

98. The method of claim 87, wherein h is a member independently selected from the integers between 1 and 3; a, b, c, d, e, f, g, i, j, k, l, m, r, s, t, and u are members independently selected from 0 and 1; n, v, w, x, and y are 0; and q, p are 1.

99. The method of claim 87, wherein a, b, c, d, f, h, j, k, l, m, n, s, u, v, w, x, and y are 0; e, g, i, r, and t are members independently selected from 0 and 1; and q,p are 1.

100. The method of claim 87, wherein a, b, c, d, e, f, g, h,j, k, l, m, n, r, s, t, u, v, w, x, and y are 0; q, p are 1; and i is independently selected from 0 and 1.

101. The method of claim 87, wherein a, b, c, d, e, f, g, h, I, j, k, l, m, r, s, t, u, v, w, x, and y are 0; and p, q are 1.

102. The method of claim 87, wherein a, b, c, d, e, f, g, h, i, j, k, l, m, and n are 0; q, p are 1; and r, s, t, u, v, w, x, and y are members independently selected from 0 and 1.

103. The method of claim 87, wherein a, b, c, d, e, f, g, h, i, r, s, t, and u are members independently selected from 0 and 1; j, k, l, m, n, v, w, x, and y are 0; and q, p are 1.

104. The method of claim 87, wherein a, b, c, d, h, j, k, l, m, r, s, t, and u are members independently selected from 0 and 1; e, f, g, are

members selected from the integers between 0 and 3; n, v, w, x, and y are 0; and q, p are 1.

105. The method of claim 87, wherein a, b, c, d, i, j, k, l, m, r, s, t, u, p and q are members independently selected from 0 and 1; e, f, g, and h are 1; and n, v, w, x, and y are 0.

106. An interferon beta peptide conjugate formed by the method of claim 87.

107. An interferon beta peptide comprising one or more glycans, having a glycoconjugate molecule covalently attached to said peptide.

108. The interferon beta peptide of claim 107, wherein said one or more glycans is a monoantennary glycan.

109. The interferon beta peptide of claim 107, wherein said one or more glycans is a biantennary glycan.

110. The interferon beta peptide of claim 107, wherein said one or more glycans is a triantennary glycan.

111. The interferon beta peptide of claim 107, wherein said one or more glycans is at least a triantennary glycan.

112. The interferon beta peptide of claim 107, wherein said one or more glycans comprises at least two glycans comprising a mixture of mono or multiantennary glycans.

113. The interferon beta peptide of claim 107, wherein said one or more glycans is selected from an **N-linked** glycan and an **O-linked** glycan.

114. The interferon beta peptide of claim 107, wherein said one or more glycans is at least two glycans selected from an **N-linked** and an **O-linked** glycan.

115. The interferon beta peptide of claim 107, wherein said peptide is expressed in a cell selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

116. The interferon beta peptide of claim 115, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell and a yeast cell.

117. A method of treating a mammal having multiple sclerosis, said method comprising administering to said mammal an interferon beta peptide having one or more glycans having a glycoconjugate molecule attached to said peptide.

118. The method of claim 117, wherein said glycoconjugate is poly(ethylene glycol).

119. The method of claim 117, wherein said mammal is a human.

L13 ANSWER 7 OF 12 USPATFULL on STN
2004:107626 Interferon alpha: remodeling and glycoconjugation of interferon alpha.
DeFrees, Shawn, North Wales, PA, UNITED STATES
Zopf, David, Wayne, PA, UNITED STATES
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US 2004082026 A1 20040429
APPLICATION: US 2003-411049 A1 20030409 (10)
PRIORITY: US 2002-407527P 20020828 (60)
US 2002-404249P 20020816 (60)
US 2002-396594P 20020717 (60)
US 2002-391777P 20020625 (60)
US 2002-387292P 20020607 (60)
US 2001-334301P 20011128 (60)
US 2001-334233P 20011128 (60)
US 2001-344692P 20011019 (60)
US 2001-328523P 20011010 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A cell-free, in vitro method of remodeling an interferon alpha peptide, said peptide having the formula: ##STR149## wherein AA is a terminal or internal amino acid residue of said peptide; X_1-X_2 is a saccharide covalently linked to said AA, wherein X_1 is a first glycosyl residue; and X_2 is a second glycosyl residue covalently linked to X_1, wherein X_1 and X_2 are selected from monosaccharyl and oligosaccharyl residues; said method comprising: (a) removing X_2 or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said interferon alpha peptide.

2. The method of claim 1, further comprising: (c) removing X_1, thereby exposing said AA; and (d) contacting said AA with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said AA, thereby remodeling said interferon alpha peptide.

3. The method of claim 1, further comprising: (e) prior to step (b), removing a group added to said saccharide during post-translational modification.

4. The method of claim 3, wherein said group is a member selected from phosphate, sulfate, carboxylate and esters thereof.

5. The method of claim 1, wherein said peptide has the formula: ##STR150## wherein Z is a member selected from O, S, NH or a crosslinker.

6. The method of claim 1, wherein at least one of said glycosyl donors comprises a modifying group.

7. The method of claim 1, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

8. The method of claim 7, wherein said modifying group is a water soluble polymer.

9. The method of claim 8, wherein said water soluble polymer comprises poly(ethylene glycol).

10. The method of claim 9, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

11. A cell-free in vitro method of remodeling an interferon alpha peptide, said peptide having the formula: ##STR151## wherein X_3, X_4, X_5, X_6, X_7, and X_{17} are independently selected monosaccharyl or oligosaccharyl residues; and a, b, c, d, e and x are independently selected from the integers 0, 1 and 2, with the proviso that at least one member selected from a, b, c, d, and e and x are 1 or 2; said method comprising: (a) removing at least one of X_3, X_4, X_5, X_6, X_7, or X_{17}, a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said interferon alpha peptide.

12. The method of claim 11, wherein said removing of step (a) produces a truncated glycan in which a, b, c, e and x are each 0.

13. The method of claim 11, wherein X_3, X_5 and X_7 are selected from the group consisting of (mannose), and $(mannose)_z-(X_8)_y$ wherein X_8 is a glycosyl moiety selected from mono- and oligo-saccharides; y is an integer selected from 0 and 1; and z is an integer between 1 and 20, wherein when z is 3 or greater, $(mannose)_z$ is selected from linear and branched structures.

14. The method of claim 11, wherein X_4 is selected from the group consisting of GlcNAc and xylose.

15. The method of claim 11, wherein X_3, X_5 and X_7 are $(mannose)_u$, wherein u is selected from the integers between 1 and 20, and when u is 3 or greater, $(mannose)_u$ is selected from linear and branched structures.

16. The method of claim 11, wherein at least one of said glycosyl donors comprises a modifying group.

17. The method of claim 16, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

18. The method of claim 17 wherein said modifying group is a water soluble polymer.

19. The method of claim 18, wherein said water soluble polymer comprises poly(ethylene glycol).

20. The method of claim 19, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

21. A cell-free in vitro method of remodeling an interferon alpha peptide comprising a glycan having the formula: ##STR152## wherein r, s, and t are integers independently selected from 0 and 1, said method comprising: (a) contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said glycan, thereby remodeling said interferon alpha peptide.

22. The method of claim 21, wherein at least one of said glycosyl donors

comprises a modifying group.

23. The method of claim 22, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

24. The method of claim 23 wherein said modifying group is a water soluble polymer.

25. The method of claim 24, wherein said water soluble polymer comprises poly(ethylene glycol).

26. The method of claim 25, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

27. The method of claim 1, wherein said peptide has the formula: ##STR153## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1.

28. The method of claim 1, wherein said peptide has the formula: ##STR154## wherein X_{11} and X_{12} are independently selected glycosyl moieties; and r and x are integers independently selected from 0 and 1.

29. The method of claim 28, wherein X_{11} and X_{12} are $(mannose)_q$, wherein q is selected from the integers between 1 and 20, and when q is three or greater $(mannose)_q$ is selected from linear and branched structures.

30. The method of claim 1, wherein said peptide has the formula: ##STR155## wherein X_{13}, X_{14}, and X_{15} are independently selected glycosyl residues; and g, h, i, j, k, and p are independently selected from the integers 0 and 1, with the proviso that at least one of g, h, i, j, k and p is 1.

31. The method of claim 30, wherein X_{14} and X_{15} are members independently selected from GlcNAc and Sia; and i and k are independently selected from the integers 0 and 1, with the proviso that at least one of i and k is 1, and when k is 1, g, h, and j are 0.

32. The method of claim 1, wherein said peptide has the formula: ##STR156## wherein X_{16} is a member selected from: ##STR157## wherein s and i are integers independently selected from 0 and 1.

33. The method of claim 1, wherein said removing utilizes a glycosidase.

34. A cell-free, in vitro method of remodeling an interferon alpha peptide having the formula: ##STR158## wherein AA is a terminal or internal amino acid residue of said peptide; X_1 is a glycosyl residue covalently linked to said AA, selected from monosaccharyl and oligosaccharyl residues; and u is an integer selected from 0 and 1, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, wherein said glycosyl donor comprises a modifying group, thereby remodeling said interferon alpha peptide.

35. The method of claim 34, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a

peptide.

36. The method of claim 35 wherein said modifying group is a water soluble polymer.

37. The method of claim 36, wherein said water soluble polymer comprises poly(ethylene glycol).

38. The method of claim 37, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

39. A covalent conjugate between an interferon alpha peptide and a modifying group that alters a property of said peptide, wherein said modifying group is covalently attached to said peptide at a preselected glycosyl or amino acid residue of said peptide via an intact glycosyl linking group.

40. The covalent conjugate of claim 39, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

41. The covalent conjugate of claim 39, wherein said modifying group and an intact glycosyl linking group precursor are linked as a covalently attached unit to said peptide via the action of an enzyme, said enzyme converting said precursor to said intact glycosyl linking group, thereby forming said conjugate.

42. The covalent conjugate of claim 39 comprising:. a first modifying group covalently linked to a first residue of said peptide via a first intact glycosyl linking group, and a second glycosyl linking group linked to a second residue of said peptide via a second intact glycosyl linking group.

43. The covalent conjugate of claim 42, wherein said first residue and said second residue are structurally identical.

44. The covalent conjugate of claim 42, wherein said first residue and said second residue have different structures.

45. The covalent conjugate of claim 42 wherein said first residue and said second residue are glycosyl residues.

46. The covalent conjugate of claim 42, wherein said first residue and said second residue are amino acid residues.

47. The covalent conjugate of claim 39, wherein said peptide is remodeled prior to forming said conjugate.

48. The covalent conjugate of claim 47, wherein the remodeled peptide is remodeled to introduce an acceptor moiety for said intact glycosyl linking group.

49. The covalent conjugate of claim 39, wherein said modifying group is a water-soluble polymer.

50. The covalent conjugate of claim 49, wherein said water-soluble polymer comprises poly(ethylene glycol).

51. The covalent conjugate of claim 39, wherein said intact glycosyl linking unit is a member selected from the group consisting of a sialic acid residue, a Gal residue, a GlcNAc residue and a GalNAc residue.

52. The covalent conjugate of claim 50, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

53. A method of forming a covalent conjugate between a polymer and a **glycosylated** or non-**glycosylated** peptide, wherein said polymer is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said polymer, said method comprising: contacting said peptide with a mixture comprising a nucleotide sugar covalently linked to said polymer and a glycosyltransferase for which said nucleotide sugar is a substrate under conditions sufficient to form said conjugate, wherein said peptide is interferon alpha.

54. The method of claim 53, wherein said polymer is a water-soluble polymer.

55. The method of claim 53, wherein said glycosyl linking group is covalently attached to a glycosyl residue covalently attached to said peptide.

56. The method of claim 53, wherein said glycosyl linking group is covalently attached to an amino acid residue of said peptide.

57. The method of claim 53, wherein said polymer comprises a member selected from the group consisting of a polyalkylene oxide and a polypeptide.

58. The method of claim 57, wherein said polyalkylene oxide is poly(ethylene glycol).

59. The method of claim 58, wherein said poly(ethylene glycol) has a degree of polymerization of from about 1 to about 20,000.

60. The method of claim 59, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 5,000.

61. The method of claim 60, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 1,000.

62. The method of claim 53, wherein said glycosyltransferase is selected from the group consisting of sialyltransferase, galactosyltransferase, glucosyltransferase, GalNAc transferase, GlcNAc transferase, fucosyltransferase, and mannosyltransferase.

63. The method of claim 53, wherein said glycosyltransferase is recombinantly produced.

64. The method of claim 63, wherein said glycosyltransferase is a recombinant prokaryotic enzyme.

65. The method of claim 63, wherein said glycosyltransferase is a recombinant eukaryotic enzyme.

66. The method of claim 53, wherein said nucleotide sugar is selected from the group consisting of UDP-glycoside, CMP-glycoside, and GDP-glycoside.

67. The method of claim 66, wherein said nucleotide sugar is selected from the group consisting of UDP-galactose, UDP-galactosamine, UDP-glucose, UDP-glucosamine, UDP-N-acetylgalactosamine,

UDP-N-acetylglucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid, CMP-NeuAc.

68. The method of claim 53, wherein said **glycosylated** peptide is partially deglycosylated prior to said contacting.

69. The method of claim 53, wherein said intact glycosyl linking group is a sialic acid residue.

70. The method of claim 53, wherein said method is performed in a cell-free **environment**.

71. The method of claim 53, wherein said covalent conjugate is isolated.

72. The method of claim 71, wherein said covalent conjugate is isolated by membrane filtration.

73. A composition for forming a conjugate between a peptide and a modified sugar, said composition comprising: an admixture of a modified sugar, a glycosyltransferase, and a peptide acceptor substrate, wherein said modified sugar has covalently attached thereto a member selected from a polymer, a therapeutic moiety and a biomolecule, wherein said peptide is interferon alpha.

74. An interferon alpha peptide remodeled by the method of claim 1.

75. A pharmaceutical composition comprising the interferon alpha peptide of claim 74.

76. An interferon alpha peptide remodeled by the method of claim 11.

77. A pharmaceutical composition comprising the interferon alpha peptide of claim 76.

78. A interferon alpha peptide remodeled by the method of claim 21.

79. A pharmaceutical composition comprising the interferon alpha peptide of claim 78.

80. An interferon alpha peptide remodeled by the method of claim 27.

81. A pharmaceutical composition comprising the interferon alpha peptide of claim 80.

82. An interferon alpha peptide remodeled by the method of claim 28.

83. A pharmaceutical composition comprising the interferon alpha peptide of claim 82.

84. An interferon alpha peptide remodeled by the method of claim 34.

85. A pharmaceutical composition comprising the interferon alpha peptide of claim 84.

86. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR159## wherein AA is a terminal or internal amino acid residue of said peptide, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said amino acid residue, wherein said glycosyl donor comprises a modifying group, thereby remodeling said peptide, wherein said peptide is interferon alpha.

87. A method of forming a conjugate between an interferon alpha peptide and a modifying group, wherein said modifying group is covalently attached to said glycopeptide through an intact glycosyl linking group, said glycopeptide comprising a glycosyl residue having a formula selected from: ##STR160## wherein a, b, c, d, i, n, o, p, q, r, s, t, u, aa, bb, cc, dd, and ee are members independently selected from 0 and 1; e, f, g, and h are members independently selected from the integers from 0 to 6; j, k, l, and m are members independently selected from the integers from 0 to 20; v, w, x, y, and z are 0; and R is a modifying group, a mannose or an oligomannose R' is H, a glycosyl residue, a modifying group, or a glycoconjugate, said method comprising: (a) contacting said glycopeptide with a member selected from a glycosyltransferase, an GalNAc transferase operating synthetically and a sialyltransferase, and a modified glycosyl donor, comprising a glycosyl moiety which is a substrate for said glycosyltransferase covalently linked to said modifying group, under conditions appropriate for the formation of said intact glycosyl linking group.

88. The method of claim 87, further comprising: (b) prior to step (a), contacting said glycopeptide with a sialidase under conditions appropriate to remove sialic acid from said glycopeptide.

89. The method of claim 87, further comprising: (c) contacting the product from step (a) with a moiety that reacts with said modifying group, thereby forming a conjugate between said intact glycosyl linking group and said moiety.

90. The method of claim 87, further comprising: (d) prior to step (a) contacting said glycopeptide with a combination of a glycosidase and a sialidase.

91. The method of claim 87, further comprising: (e) prior to step (a), contacting said glycopeptide with an endoglycanase under conditions appropriate to cleave a glycosyl moiety from said glycopeptide.

92. The method of claim 87, further comprising: (f) prior to step (a), contacting said glycopeptide with N-acetylglucosamine transferase and a GlcNAc donor under conditions appropriate to transfer GlcNAc to said glycopeptide.

93. The method of claim 87, further comprising: (g) prior to step (a), contacting said glycopeptide with a galactosyl transferase and a galactose donor under conditions appropriate to transfer galactose to said product.

94. The method of claim 87, further comprising: (h) prior to step (b), contacting said glycopeptide with endoglycanase under conditions appropriate to cleave a glycosyl moiety from said glycopeptide.

95. The method of claim 87, further comprising: (i) prior to step (a), contacting said glycopeptide with a mannosidase under conditions appropriate to remove mannose from said glycopeptide.

96. The method of claim 87, further comprising: (j) contacting the product of step (a) with a sialyltransferase and a sialic acid donor under conditions appropriate to transfer sialic acid to said product.

97. The method of claim 87, wherein said modifying group is a member selected from a polymer, a toxin, a radioisotope, a therapeutic moiety and a glycoconjugate.

98. The method of claim 87, wherein a, b, c, d, aa, and bb are 1; e, f, g, and h are members independently selected from the integers from 1 to 4; i, j, k, l, m, r, s, t, u, and cc are members independently selected from 0 and 1; and n, o, p, q, v, w, x, y, z, dd, and ee are 0.

99. The method of claim 87, wherein a, b, c, d, f, h, j, k, l, m, n, o, p, q, s, u, v, w, x, y, z, cc, dd, and ee are 0; e, g, i, r, and t are members independently selected from 0 and 1; and aa and bb are 1.

100. The method of claim 87, wherein a, b, c, d, e, f, g, i, j, k, l, m, r, s, t, and u are members independently selected from 0 and 1; h is a member independently selected from the integers from 1 to 3; dd, v, w, x, and y are 0; and aa and bb are 1.

101. The method of claim 87, wherein a, b, c, d, f, h, j, k, l, m, s, u, v, w, x, y, and dd are 0; e, g, i, r, and t are members independently selected from 0 and 1; and aa and bb are 1.

102. The method of claim 87, wherein a, b, c, d, e, f, g, h, i, j, k, l, m, and dd are 0; r, s, t, u, v, w, x, and y are members independently selected from 0 and 1; and aa and bb are 1.

103. The method of claim 87, wherein a, b, c, d, e, f, g, h, i, r, s, t, and u are members independently selected from 0 and 1; j, k, l, m, v, w, x, y, and dd are 0; and aa and bb are 1.

104. The method of claim 87, wherein a, b, c, d, e, f, g, i, j, k, l, m, r, s, t, and u are members independently selected from 0 and 1; h is a member independently selected from the integers from 1 to 3; v, w, x, y, and dd are 0; and aa and bb are 1.

105. The method of claim 87, wherein a, b, c, d, f, h, j, k, l, m, s, u, v, w, x, y, and dd are 0; e, g, i, r, and t are members independently selected from 0 and 1; and aa and bb are 1.

106. The method of claim 87, wherein n, o, and p are members independently selected from 0 and 1; q is 1; and z, cc, and ee are 0.

107. The method of claim 87, wherein n and q are members independently selected from 0 and 1; and o, p, z, cc, and ee are 0.

108. The method of claim 87, wherein n is 0 or 1; q is 1; and o, p, z, cc, and ee are 0.

109. The method of claim 87, wherein n, o, p, and f are members independently selected from 0 and 1; q is 1; and z and ee are 0.

110. The method of claim 87, wherein n, o, p, and q are members independently selected from 0 and 1; and z, cc, and ee are 0.

111. The method of claim 87, wherein n and q are members independently selected from 0 and 1; and o, p, z, cc, and ee are 0.

112. The method of claim 87, wherein n, o, p, q, z, cc, and ee are 0.

113. An interferon alpha peptide conjugate formed by the method of claim 87.

114. An interferon alpha peptide comprising one or more glycans, having a glycoconjugate molecule covalently attached to said peptide.

115. The interferon alpha peptide of claim 114, wherein said one or more

glycans is a monoantennary glycan.

116. The interferon alpha peptide of claim 114, wherein said one or more glycans is a biantennary glycan.

117. The interferon alpha peptide of claim 114, wherein said one or more glycans is a triantennary glycan.

118. The interferon alpha peptide of claim 114, wherein said one or more glycans is at least a triantennary glycan.

119. The interferon alpha peptide of claim 114, wherein said one or more glycans comprises at least two glycans comprising a mixture of mono or multiantennary glycans.

120. The interferon alpha peptide of claim 114, wherein said one or more glycans is selected from an **N-linked** glycan and an **O-linked** glycan.

121. The interferon alpha peptide of claim 114, wherein said one or more glycans is at least two glycans selected from an **N-linked** and an **O-linked** glycan.

122. The interferon alpha peptide of claim 114, wherein said peptide is expressed in a cell selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

123. The interferon alpha peptide of claim 122, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell and a yeast cell.

124. A method of treating a mammal having hepatitis C, said method comprising administering to said mammal an interferon alpha peptide having one or more glycans having a glycoconjugate molecule attached to said peptide.

125. The method of claim 124, wherein said glycoconjugate is poly(ethylene glycol).

126. The method of claim 124, wherein said mammal is a human.

L13 ANSWER 8 OF 12 USPATFULL on STN
2004:101966 Granulocyte colony stimulating factor: remodeling and
glycoconjugation of G-CSF.
DeFrees, Shawn, North Wales, PA, UNITED STATES
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US 2004077836 A1 20040422
APPLICATION: US 2003-410962 A1 20030409 (10)
PRIORITY: US 2002-407527P 20020828 (60)
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US 2001-334301P 20011128 (60)
US 2001-334233P 20011128 (60)
US 2001-344692P 20011019 (60)
US 2001-328523P 20011010 (60)

DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. A cell-free, in vitro method of remodeling a granulocyte colony stimulating factor (G-CSF) peptide, said peptide having the formula: ##STR150## wherein AA is a terminal or internal amino acid residue of said peptide; X_1-X_2 is a saccharide covalently linked to said AA, wherein X_1 is a first glycosyl residue; and X_2 is a second glycosyl residue covalently linked to X_1, wherein X_1 and X_2 are selected from monosaccharyl and oligosaccharyl residues; said method comprising: (a) removing X_2 or a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, thereby remodeling said G-CSF peptide.

2. The method of claim 1, further comprising: (c) removing X_1, thereby exposing said AA; and (d) contacting said AA with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said AA, thereby remodeling said G-CSF peptide.

3. The method of claim 1, further comprising: (e) prior to step (b), removing a group added to said saccharide during post-translational modification.

4. The method of claim 3, wherein said group is a member selected from phosphate, sulfate, carboxylate and esters thereof.

5. The method of claim 1, wherein said peptide has the formula: ##STR151## wherein Z is a member selected from O, S, NH and a crosslinker.

6. The method of claim 1, wherein at least one of said glycosyl donors comprises a modifying group.

7. The method of claim 1, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

8. The method of claim 7, wherein said modifying group is a water soluble polymer.

9. The method of claim 8, wherein said water soluble polymer comprises poly(ethylene glycol).

10. The method of claim 9, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

11. A cell-free in vitro method of remodeling a G-CSF peptide, said peptide having the formula: ##STR152## wherein X_3, X_4, X_5, X_6, X_7, and X_{17} are independently selected monosaccharyl or oligosaccharyl residues; and a, b, c, d, e and x are independently selected from the integers 0, 1 and 2, with the proviso that at least one member selected from a, b, c, d, and e and x are 1 or 2; said method comprising: (a) removing at least one of X_3, X_4, X_5, X_6, X_7, or X_{17}, a saccharyl subunit thereof from said peptide, thereby forming a truncated glycan; and (b) contacting said truncated glycan with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer

said at least one glycosyl donor to said truncated glycan, thereby remodeling said G-CSF peptide.

12. The method of claim 11, wherein said removing of step (a) produces a truncated glycan in which a, b, c, e and x are each 0.

13. The method of claim 11, wherein X_3, X_5, and X_7, are selected from the group consisting of $(mannose)_z$ and $(mannose)_z-(X_8)_y$ wherein X_8 is a glycosyl moiety selected from mono- and oligo-saccharides; y is an integer selected from 0 and 1; and z is an integer between 1 and 20, wherein when z is 3 or greater, $(mannose)_z$ is selected from linear and branched structures.

14. The method of claim 11, wherein X_4 is selected from the group consisting of GlcNAc and xylose.

15. The method of claim 11, wherein X_3, X_5, and X_7 are $(mannose)_u$, wherein u is selected from the integers between 1 and 20, and when u is 3 or greater, $(mannose)_u$ is selected from linear and branched structures.

16. The method of claim 11, wherein at least one of said glycosyl donors comprises a modifying group.

17. The method of claim 16, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

18. The method of claim 17 wherein said modifying group is a water soluble polymer.

19. The method of claim 18, wherein said water soluble polymer comprises poly(ethylene glycol).

20. The method of claim 19, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

21. A cell-free in vitro method of remodeling a G-CSF peptide comprising a glycan having the formula: ##STR153## wherein r, s, and t are integers independently selected from 0 and 1, said method comprising: (a) contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said glycan, thereby remodeling said G-CSF peptide.

22. The method of claim 21, wherein at least one of said glycosyl donors comprises a modifying group.

23. The method of claim 22, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

24. The method of claim 23 wherein said modifying group is a water soluble polymer.

25. The method of claim 24, wherein said water soluble polymer comprises poly(ethylene glycol).

26. The method of claim 25, wherein said poly(ethylene glycol) has a

molecular weight distribution that is essentially homodisperse.

27. The method of claim 1, wherein said peptide has the formula: ##STR154## wherein X_9 and X_{10} are independently selected monosaccharyl or oligosaccharyl residues; and m, n and f are integers selected from 0 and 1.

28. The method of claim 1, wherein said peptide has the formula: ##STR155## wherein X_{11} and X_{12} are independently selected glycosyl moieties; and r and x are integers independently selected from 0 and 1.

29. The method of claim 28, wherein X_{11} and X_{12} are $(mannose)_q$, wherein q is selected from the integers between 1 and 20, and when q is three or greater, $(mannose)_q$ is selected from linear and branched structures.

30. The method of claim 1, wherein said peptide has the formula: ##STR156## wherein X_{13}, X_{14}, and X_{15} are independently selected glycosyl residues; and g, h, i, j, k, and p are independently selected from the integers 0 and 1, with the proviso that at least one of g, h, i, j, k and p is 1.

31. The method of claim 30, wherein X_{14} and X_{15} are members independently selected from GlcNAc and Sia; and i and k are independently selected from the integers 0 and 1, with the proviso that at least one of i and k is 1 and if k is 1, g, h and j are 0.

32. The method of claim 1, wherein said peptide has the formula: ##STR157## wherein X_{16} is a member selected from: ##STR158## wherein s and i are integers independently selected from 0 and 1.

33. The method of claim 1, wherein said removing utilizes a glycosidase.

34. A cell-free, in vitro method of remodeling a G-CSF peptide having the formula: ##STR159## wherein AA is a terminal or internal amino acid residue of said peptide; X_1 is a glycosyl residue covalently linked to said AA, selected from monosaccharyl and oligosaccharyl residues; and u is an integer selected from 0 and 1, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said truncated glycan, wherein said glycosyl donor comprises a modifying group, thereby remodeling said G-CSF peptide.

35. The method of claim 34, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

36. The method of claim 35 wherein said modifying group is a water soluble polymer.

37. The method of claim 36, wherein said water soluble polymer comprises poly(ethylene glycol).

38. The method of claim 37, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

39. A covalent conjugate between a G-CSF peptide and a modifying group that alters a property of said peptide, wherein said modifying group is covalently attached to said peptide at a preselected glycosyl or amino

acid residue of said peptide via an intact glycosyl linking group.

40. The covalent conjugate of claim 39, wherein said modifying group is a member selected from the group consisting of a polymer, a therapeutic moiety, a detectable label, a reactive linker group, a targeting moiety, and a peptide.

41. The covalent conjugate of claim 39, wherein said modifying group and an intact glycosyl linking group precursor are linked as a covalently attached unit to said peptide via the action of an enzyme, said enzyme converting said precursor to said intact glycosyl linking group, thereby forming said conjugate.

42. The covalent conjugate of claim 39 comprising: a first modifying group covalently linked to a first residue of said peptide via a first intact glycosyl linking group, and a second glycosyl linking group linked to a second residue of said peptide via a second intact glycosyl linking group.

43. The covalent conjugate of claim 42, wherein said first residue and said second residue are structurally identical.

44. The covalent conjugate of claim 42, wherein said first residue and said second residue have different structures.

45. The covalent conjugate of claim 42 wherein said first residue and said second residue are glycosyl residues.

46. The covalent conjugate of claim 42, wherein said first residue and said second residue are amino acid residues.

47. The covalent conjugate of claim 39, wherein said peptide is remodeled prior to forming said conjugate.

48. The covalent conjugate of claim 47, wherein the remodeled peptide is remodeled to introduce an acceptor moiety for said intact glycosyl linking group.

49. The covalent conjugate of claim 39, wherein said modifying group is a water-soluble polymer.

50. The covalent conjugate of claim 49, wherein said water-soluble polymer comprises poly(ethylene glycol).

51. The covalent conjugate of claim 39, wherein said intact glycosyl linking unit is a member selected from the group consisting of a sialic acid residue, a Gal residue, a GlcNAc residue and a GalNAc residue.

52. The covalent conjugate of claim 50, wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

53. A method of forming a covalent conjugate between a polymer and a **glycosylated** or non-**glycosylated** peptide, wherein said polymer is conjugated to said peptide via an intact glycosyl linking group interposed between and covalently linked to both said peptide and said polymer, said method comprising: contacting said peptide with a mixture comprising a nucleotide sugar covalently linked to said polymer and a glycosyltransferase for which said nucleotide sugar is a substrate under conditions sufficient to form said conjugate, wherein said peptide is G-CSF.

54. The method of claim 53, wherein said polymer is a water-soluble polymer.

55. The method of claim 53, wherein said glycosyl linking group is covalently attached to a glycosyl residue covalently attached to said peptide.

56. The method of claim 53, wherein said glycosyl linking group is covalently attached to an amino acid residue of said peptide.

57. The method of claim 53, wherein said polymer comprises a member selected from the group consisting of a polyalkylene oxide and a polypeptide.

58. The method of claim 57, wherein said polyalkylene oxide is poly(ethylene glycol).

59. The method of claim 58, wherein said poly(ethylene glycol) has a degree of polymerization of from about 1 to about 20,000.

60. The method of claim 59, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 5,000.

61. The method of claim 60, wherein said polyethylene glycol has a degree of polymerization of from about 1 to about 1,000.

62. The method of claim 53, wherein said glycosyltransferase is selected from the group consisting of sialyltransferase, galactosyltransferase, glucosyltransferase, GalNAc transferase, GlcNAc transferase, fucosyltransferase, and mannosyltransferase.

63. The method of claim 53, wherein said glycosyltransferase is recombinantly produced.

64. The method of claim 63, wherein said glycosyltransferase is a recombinant prokaryotic enzyme.

65. The method of claim 63, wherein said glycosyltransferase is a recombinant eukaryotic enzyme.

66. The method of claim 53, wherein said nucleotide sugar is selected from the group consisting of UDP-glycoside, CMP-glycoside, and GDP-glycoside.

67. The method of claim 66, wherein said nucleotide sugar is selected from the group consisting of UDP-galactose, UDP-galactosamine, UDP-glucose, UDP-glucosamine, UDP-N-acetylgalactosamine, UDP-N-acetylglucosamine, GDP-mannose, GDP-fucose, CMP-sialic acid, CMP-NeuAc.

68. The method of claim 53, wherein said **glycosylated** peptide is partially deglycosylated prior to said contacting.

69. The method of claim 53, wherein said intact glycosyl linking group is a sialic acid residue.

70. The method of claim 53, wherein said method is performed in a cell-free **environment**.

71. The method of claim 53, wherein said covalent conjugate is isolated.

72. The method of claim 71, wherein said covalent conjugate is isolated

by membrane filtration.

73. A composition for forming a conjugate between a peptide and a modified sugar, said composition comprising: an admixture of a modified sugar, a glycosyltransferase, and a peptide acceptor substrate, wherein said modified sugar has covalently attached thereto a member selected from a polymer, a therapeutic moiety and a biomolecule, wherein said peptide is G-CSF.

74. A G-CSF peptide remodeled by the method of claim 1.

75. A pharmaceutical composition comprising the G-CSF peptide of claim 74.

76. A G-CSF peptide remodeled by the method of claim 11.

77. A pharmaceutical composition comprising the G-CSF peptide of claim 76.

78. A G-CSF peptide remodeled by the method of claim 21.

79. A pharmaceutical composition comprising the G-CSF peptide of claim 78.

80. A G-CSF peptide remodeled by the method of claim 27.

81. A pharmaceutical composition comprising the G-CSF peptide of claim 80.

82. A G-CSF peptide remodeled by the method of claim 28.

83. A pharmaceutical composition comprising the G-CSF peptide of claim 82.

84. A G-CSF peptide remodeled by the method of claim 34.

85. A pharmaceutical composition comprising the G-CSF peptide of claim 84.

86. A cell-free, in vitro method of remodeling a peptide having the formula: ##STR160## wherein AA is a terminal or internal amino acid residue of said peptide, said method comprising: contacting said peptide with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said amino acid residue, wherein said glycosyl donor comprises a modifying group, thereby remodeling said peptide, wherein said peptide is G-CSF.

87. A method of forming a conjugate between a G-CSF peptide and a modifying group, wherein said modifying group is covalently attached to said G-CSF peptide through an intact glycosyl linking group, said G-CSF peptide comprising a glycosyl residue having the formula: ##STR161## wherein a, b, c, and e are members independently selected from 0 and 1; d is 0; and R is a modifying group, a sialic acid or an oligosialic acid, said method comprising: (a) contacting said G-CSF peptide with a glycosyltransferase and a modified glycosyl donor, comprising a glycosyl moiety which is a substrate for said glycosyltransferase covalently linked to said modifying group, under conditions appropriate for the formation of said intact glycosyl linking group.

88. The method of claim 87, further comprising: (b) prior to step (a), contacting said G-CSF peptide with a sialidase under conditions

appropriate to remove sialic acid from said G-CSF peptide.

89. The method of claim 87, further comprising: (c) prior to step (a), contacting said G-CSF peptide with a galactosyl transferase and a galactose donor under conditions appropriate to transfer said galactose to said G-CSF peptide.

90. The method of claim 87, further comprising: (d) contacting the product from step (a) with a moiety that reacts with said modifying group, thereby forming a conjugate between said intact glycosyl linking group and said moiety.

91. The method of claim 87, further comprising: (e) prior to step (a), contacting said G-CSF peptide with N-acetylgalactosamine transferase and a GalNAc donor under conditions appropriate to transfer GalNAc to said G-CSF peptide.

92. The method of claim 87, further comprising: (f) prior to step (a), contacting said G-CSF peptide with endo-N-acetylgalactosaminidase operating synthetically and a GalNAc donor under conditions appropriate to transfer GalNAc to said G-CSF peptide.

93. The method of claim 87, wherein said modifying group is a member selected from a polymer, a toxin, a radioisotope, a therapeutic moiety and a glycoconjugate.

94. The method of claim 87, wherein a, b, c, and e are 0.

95. The method of claim 87, wherein a and e are members independently selected from 0 and 1; and b, c, and d are 0.

96. The method of claim 87, wherein a, b, c, d, and e are members independently selected from 0 and 1.

97. A G-CSF peptide conjugate formed by the method of claim 87.

98. A G-CSF peptide comprising one or more glycans, having a glycoconjugate molecule covalently attached to said peptide.

99. The G-CSF peptide of claim 98, wherein said one or more glycans is a monoantennary glycan.

100. The G-CSF peptide of claim 98, wherein said one or more glycans is a biantennary glycan.

101. The G-CSF peptide of claim 98, wherein said one or more glycans is a triantennary glycan.

102. The G-CSF peptide of claim 98, wherein said one or more glycans is at least a triantennary glycan.

103. The G-CSF peptide of claim 98, wherein said one or more glycans comprises at least two glycans comprising a mixture of mono or multiantennary glycans.

104. The G-CSF peptide of claim 98, wherein said one or more glycans is selected from an **N-linked** glycan and an **O-linked** glycan.

105. The G-CSF peptide of claim 98, wherein said one or more glycans is at least two glycans selected from an **N-linked** and an **O-linked** glycan.

106. The G-CSF peptide of claim 98, wherein said peptide is expressed in

a cell selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

107. The G-CSF peptide of claim 106, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell and a yeast cell.

108. A method of treating a mammal having a disease selected from the group consisting of an infectious disease, acute myeloid leukemia, non-myeloid cancer, chronic or persistent neutropenia, said method comprising administering to said mammal a G-CSF peptide having one or more glycans having a glycoconjugate molecule attached to said peptide.

109. The method of claim 108, wherein said infectious disease is selected from the group consisting of a bacterial and a viral disease.

110. The method of claim 108, wherein said glycoconjugate molecule is poly(ethylene glycol).

111. The method of claim 108, wherein said mammal is a human.

L13 ANSWER 9 OF 12 USPATFULL on STN
2004:7370 Phospholipases, nucleic acids encoding them and methods for making and using them.
Gramatikova, Svetlana, San Diego, CA, UNITED STATES
Hazlewood, Geoff, San Diego, CA, UNITED STATES
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Diversa Corporation, San Diego, CA, UNITED STATES (U.S. corporation)
US 2004005604 A1 20040108
APPLICATION: US 2003-421654 A1 20030421 (10)
PRIORITY: US 2002-374313P 20020419 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An isolated or recombinant nucleic acid comprising a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, over a region of at least about 100 residues, wherein the nucleic acid encodes at least one polypeptide having a phospholipase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

2. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63% or 64%.

3. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%,76%,77%,78%,79%,80%,81%,82%,83%,84%,85%,86%,87%,88%,8 9%,90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or is 100% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7,

SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105.

4. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more residues, or the full length of a gene or a transcript.

5. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence comprises a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105.

6. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence encodes a polypeptide having a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106.

7. The isolated or recombinant nucleic acid of claim 1, wherein the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall-p blastp-d "nr pataa"-F F, and all other options are set to default.

8. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage.

9. The isolated or recombinant nucleic acid of claim 8, wherein the phospholipase activity comprises catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil.

10. The isolated or recombinant nucleic acid of claim 8, wherein the vegetable oil phospholipid comprises an oilseed phospholipid.

11. The isolated or recombinant nucleic acid of claim 1, wherein the

phospholipase activity comprises a phospholipase C (PLC) activity.

12. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase A (PLA) activity.

13. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase B (PLB) activity.

14. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a phospholipase D (PLD) activity.

15. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase D activity comprises a phospholipase D1 or a phospholipase D2 activity.

16. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises hydrolysis of a glycoprotein.

17. The isolated or recombinant nucleic acid of claim 16, wherein the glycoprotein comprises a potato tuber.

18. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises a patatin enzymatic activity.

19. The isolated or recombinant nucleic acid of claim 18, wherein the phospholipase activity comprises a lipid acyl hydrolase (LAH) activity.

20. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity is thermostable.

21. The isolated or recombinant nucleic acid of claim 20, wherein the polypeptide retains a phospholipase activity under conditions comprising a temperature range of between about 37° C. to about 95° C., or between about 55° C. to about 85° C., or between about 70° C. to about 75° C., or between about 70° C. to about 95° C., or between about 90° C. to about 95° C.

22. The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity is thermotolerant.

23. The isolated or recombinant nucleic acid of claim 22, wherein the polypeptide retains a phospholipase activity after exposure to a temperature in the range from greater than 37° C. to about 95° C., from greater than 55° C. to about 85° C., or between about 70° C. to about 75° C., or from greater than 90° C. to about 95° C.

24. An isolated or recombinant nucleic acid, wherein the nucleic acid comprises a sequence that hybridizes under stringent conditions to a nucleic acid comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, wherein the nucleic acid encodes a polypeptide having a phospholipase

activity.

25. The isolated or recombinant nucleic acid of claim 24, wherein the nucleic acid is at least about 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more residues in length or the full length of the gene or transcript.

26. The isolated or recombinant nucleic acid of claim 24, wherein the stringent conditions include a wash step comprising a wash in 0.2xSSC at a temperature of about 65° C. for about 15 minutes.

27. A nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a phospholipase activity, wherein the probe comprises at least 10 consecutive bases of a sequence comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, wherein the probe identifies the nucleic acid by binding or hybridization.

28. The nucleic acid probe of claim 27, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.

29. A nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a phospholipase activity, wherein the probe comprises a nucleic acid comprising at least about 10 consecutive residues of SEQ ID NO:1; SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

30. The nucleic acid probe of claim 29, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.

31. An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a phospholipase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

32. The amplification primer pair of claim 29, wherein a member of the amplification primer sequence pair comprises an oligonucleotide

comprising at least about 10 to 50 consecutive bases, or about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive bases of the sequence.

33. An amplification primer pair, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 or more residues of the complementary strand of the first member.

34. A phospholipase-encoding nucleic acid generated by amplification of a polynucleotide using an amplification primer pair as set forth in claim 33.

35. The phospholipase-encoding nucleic acid of claim 34, wherein the amplification is by polymerase chain reaction (PCR).

36. The phospholipase-encoding nucleic acid of claim 34, wherein the nucleic acid generated by amplification of a gene library.

37. The phospholipase-encoding nucleic acid of claim 34, wherein the gene library is an **environmental** library.

38. An isolated or recombinant phospholipase encoded by a phospholipase-encoding nucleic acid as set forth in claim 34.

39. A method of amplifying a nucleic acid encoding a polypeptide having a phospholipase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

40. A method for making a phospholipase comprising amplification of a nucleic acid with an amplification primer pair as set forth in claim 33 and expression of the amplified nucleic acid.

41. An expression cassette comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

42. A vector comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

43. A cloning vehicle comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

44. The cloning vehicle of claim 43, wherein the viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector.

45. The cloning vehicle of claim 43, comprising a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

46. A transformed cell comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

47. A transformed cell comprising an expression cassette as set forth in claim 41.

48. The transformed cell of claim 47, wherein the cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell.

49. A transgenic non-human animal comprising a sequence as set forth in claim 1 or claim 24.

50. The transgenic non-human animal of claim 49, wherein the animal is a mouse.

51. A transgenic plant comprising a sequence as set forth in claim 1 or claim 24.

52. The transgenic plant of claim 51, wherein the plant is a corn plant, a sorghum plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant, a grass, a cottonseed, a palm, a sesame plant, a peanut plant, a sunflower plant or a tobacco plant.

53. A transgenic seed comprising a sequence as set forth in claim 1 or claim 24.

54. The transgenic seed of claim 53, wherein the seed is a corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a rice, a barley, a peanut, a cottonseed, a palm, a peanut, a sesame seed, a sunflower seed or a tobacco plant seed.

55. An antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

56. The antisense oligonucleotide of claim 55, wherein the antisense oligonucleotide is between about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 bases in length.

57. A method of inhibiting the translation of a phospholipase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24.

58. A double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of a sequence as set forth in claim 1 or claim 24.

59. The double-stranded inhibitory RNA (RNAi) molecule of claim 58, wherein the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length.

60. A method of inhibiting the expression of a phospholipase in a cell comprising administering to the cell or expressing in the cell a double-stranded inhibitory RNA (iRNA), wherein the RNA comprises a subsequence of a sequence as set forth in claim 1 or claim 24.

61. An isolated or recombinant polypeptide (i) having at least 50% sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or, (ii) encoded by a nucleic acid having at least 50% sequence identity to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105 over a region of at least about 100 residues, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or encoded by a nucleic acid capable of hybridizing under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:1, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105.

62. The isolated or recombinant polypeptide of claim 61, wherein the sequence identity is over a region of at least about at least about 51%, 52%, 53%, 54%, 55%, 56%,57%,58%,59%,60%,61%,62%,63%,64%,65%,66%,67%,68%, 69%,70%,71%, 72%,73%,74%,75%,76%,77%,78%,79%,80%,81%,82%,83%,84%,85%,86% ,87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or is 100% sequence identity.

63. The isolated or recombinant polypeptide of claim 61, wherein the sequence identity is over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050 or more residues, or the full length of an enzyme.

64. The isolated or recombinant polypeptide of claim 61, wherein the polypeptide has a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ

ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106.

65. The isolated or recombinant polypeptide of claim 61, wherein the polypeptide has a phospholipase activity.

66. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage.

67. The isolated or recombinant polypeptide of claim 66, wherein the phospholipase activity comprises catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil.

68. The isolated or recombinant polypeptide of claim 67, wherein the vegetable oil phospholipid comprises an oilseed phospholipid.

69. The isolated or recombinant polypeptide of claim 67, wherein the vegetable oil phospholipid is derived from a plant oil, a high phosphorous oil, a soy oil, a canola oil, a palm oil, a cottonseed oil, a corn oil, a palm kernel-derived phospholipid, a coconut oil, a peanut oil, a sesame oil, a fish oil, an algae phospholipid, a sunflower oil, an essential oil, a fruit seed oil, a grapeseed phospholipid, an apricot phospholipid, or a borage phospholipid.

70. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase C (PLC) activity.

71. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase A (PLA) activity.

72. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase a activity comprises a phospholipase A1 or phospholipase A2 activity.

73. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a phospholipase D (PLD) activity.

74. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase D activity comprises a phospholipase D1 or a phospholipase D2 activity.

75. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises hydrolysis of a glycoprotein.

76. The isolated or recombinant polypeptide of claim 68, wherein the glycoprotein comprises a potato tuber.

77. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a patatin enzymatic activity.

78. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a lipid acyl hydrolase (LAH) activity.

79. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity is thermostable.

80. The isolated or recombinant polypeptide of claim 79, wherein the polypeptide retains a phospholipase activity under conditions comprising a temperature range of between about 37° C. to about 95° C., between about 55° C. to about 85° C., between about 70° C. to about 95° C., between about 70° C. to about 75° C., or between about 90° C. to about 95° C.

81. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity is thermotolerant.

82. The isolated or recombinant polypeptide of claim 81, wherein the polypeptide retains a phospholipase activity after exposure to a temperature in the range from greater than 37° C. to about 95° C., from greater than 55° C. to about 85° C., between about 70° C. to about 75° C., or from greater than 90° C. to about 95° C.

83. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 61 and lacking a signal sequence.

84. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 61 and having a heterologous signal sequence.

85. The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises a specific activity at about 37° C. in the range from about 100 to about 1000 units per milligram of protein, from about 500 to about 750 units per milligram of protein, from about 500 to about 1200 units per milligram of protein, or from about 750 to about 1000 units per milligram of protein.

86. The isolated or recombinant polypeptide of claim 81, wherein the thermotolerance comprises retention of at least half of the specific activity of the phospholipase at 37° C. after being heated to an elevated temperature.

87. The isolated or recombinant polypeptide of claim 81, wherein the thermotolerance comprises retention of specific activity at 37° C. in the range from about 500 to about 1200 units per milligram of protein after being heated to an elevated temperature.

88. The isolated or recombinant polypeptide of claim 61, wherein the polypeptide comprises at least one **glycosylation** site.

89. The isolated or recombinant polypeptide of claim 88, wherein the **glycosylation** is an **N-linked glycosylation**.

90. The isolated or recombinant polypeptide of claim 89, wherein the polypeptide is **glycosylated** after being expressed in an P. pastoris or an S. pombe.

91. The isolated or recombinant polypeptide of claim 65, wherein the polypeptide retains a phospholipase activity under conditions comprising about pH 6.5, pH 6.0, pH 5.5, 5.0, pH 4.5 or 4.0.

92. The isolated or recombinant polypeptide of claim 65, wherein the polypeptide retains a phospholipase activity under conditions comprising about pH 7.5, pH 8.0, pH 8.5,pH 9, pH 9.5, pH 10 or pH 10.5.

93. A protein preparation comprising a polypeptide as set forth in claim 61, wherein the protein preparation comprises a liquid, a solid or a gel.

94. A heterodimer comprising a polypeptide as set forth in claim 61 and a second domain.

95. The heterodimer of claim 94, wherein the second domain is a polypeptide and the heterodimer is a fusion protein.

96. The heterodimer of claim 94, wherein the second domain is an epitope or a tag.

97. A homodimer comprising a polypeptide as set forth in claim 61.

98. An immobilized polypeptide, wherein the polypeptide comprises a sequence as set forth in claim 61, or a subsequence thereof.

99. The immobilized polypeptide of claim 98, wherein the polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.

100. An array comprising an immobilized polypeptide as set forth in claim 61.

101. An array comprising an immobilized nucleic acid as set forth in claim 1 or claim 24.

102. An isolated or recombinant antibody that specifically binds to a polypeptide as set forth in claim 61.

103. The isolated or recombinant antibody of claim 102, wherein the antibody is a monoclonal or a polyclonal antibody.

104. A hybridoma comprising an antibody that specifically binds to a polypeptide as set forth in claim 61.

105. A method of isolating or identifying a polypeptide with a phospholipase activity comprising the steps of: (a) providing an antibody as set forth in claim 102; (b) providing a sample comprising polypeptides; and (c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having a phospholipase activity.

106. A method of making an anti-phospholipase antibody comprising administering to a non-human animal a nucleic acid as set forth in claim 1 or claim 24 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-phospholipase antibody.

107. A method of making an anti-phospholipase antibody comprising administering to a non-human animal a polypeptide as set forth in claim 61 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-phospholipase antibody.

108. A method of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid operably linked to a promoter, wherein the nucleic acid comprises a sequence as set forth in claim 1 or claim 24; and (b) expressing the nucleic acid of step (a) under

conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.

109. The method of claim 108, further comprising transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.

110. A method for identifying a polypeptide having a phospholipase activity comprising the following steps: (a) providing a polypeptide as set forth in claim 65; (b) providing a phospholipase substrate; and (c) contacting the polypeptide with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a phospholipase activity.

111. A method for identifying a phospholipase substrate comprising the following steps: (a) providing a polypeptide as set forth in claim 65; (b) providing a test substrate; and (c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a phospholipase substrate.

112. A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps: (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid has a sequence as set forth in claim 1 or claim 24; (b) providing a test compound; (c) contacting the polypeptide with the test compound; and (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

113. A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps: (a) providing a polypeptide as set forth in claim 61; (b) providing a test compound; (c) contacting the polypeptide with the test compound; and (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

114. A method for identifying a modulator of a phospholipase activity comprising the following steps: (a) providing a polypeptide as set forth in claim 65; (b) providing a test compound; (c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the phospholipase, wherein a change in the phospholipase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the phospholipase activity.

115. The method of claim 114, wherein the phospholipase activity is measured by providing a phospholipase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product.

116. The method of claim 115, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of phospholipase activity.

117. The method of claim 115, wherein an increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of phospholipase activity.

118. A computer system comprising a processor and a data storage device wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 61, a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

119. The computer system of claim 118, further comprising a sequence comparison algorithm and a data storage device having at least one reference sequence stored thereon.

120. The computer system of claim 119, wherein the sequence comparison algorithm comprises a computer program that indicates polymorphisms.

121. The computer system of claim 119, further comprising an identifier that identifies one or more features in said sequence.

122. A computer readable medium having stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 61; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

123. A method for identifying a feature in a sequence comprising the steps of: (a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 61; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) identifying one or more features in the sequence with the computer program.

124. A method for comparing a first sequence to a second sequence comprising the steps of: (a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 61 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) determining differences between the first sequence and the second sequence with the computer program.

125. The method of claim 124, wherein the step of determining differences between the first sequence and the second sequence further comprises the step of identifying polymorphisms.

126. The method of claim 124, further comprising an identifier that identifies one or more features in a sequence.

127. The method of claim 126, comprising reading the first sequence using a computer program and identifying one or more features in the sequence.

128. A method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an **environmental** sample comprising the steps of: (a) providing an amplification primer sequence pair as set forth in claim 33; (b) isolating a nucleic acid from the

environmental sample or treating the **environmental** sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and, (c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the **environmental** sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an **environmental** sample.

129. The method of claim 128, wherein each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, or a subsequence thereof.

130. A method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an **environmental** sample comprising the steps of: (a) providing a polynucleotide probe comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof; (b) isolating a nucleic acid from the **environmental** sample or treating the **environmental** sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a); (c) combining the isolated nucleic acid or the treated **environmental** sample of step (b) with the polynucleotide probe of step (a); and (d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an **environmental** sample.

131. The method of claim 128 or claim 130, wherein the **environmental** sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample.

132. The method of claim 131, wherein the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

133. A method of generating a variant of a nucleic acid encoding a polypeptide with a phospholipase activity comprising the steps of: (a) providing a template nucleic acid comprising a sequence as set forth in claim 1 or claim 24; and (b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

134. The method of claim 133, further comprising expressing the variant nucleic acid to generate a variant phospholipase polypeptide.

135. The method of claim 133, wherein the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof.

136. The method of claim 133, wherein the modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

137. The method of claim 133, wherein the method is iteratively repeated until a phospholipase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced.

138. The method of claim 137, wherein the variant phospholipase polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature.

139. The method of claim 137, wherein the variant phospholipase polypeptide has increased **glycosylation** as compared to the phospholipase encoded by a template nucleic acid.

140. The method of claim 137, wherein the variant phospholipase polypeptide has a phospholipase activity under a high temperature, wherein the phospholipase encoded by the template nucleic acid is not active under the high temperature.

141. The method of claim 133, wherein the method is iteratively repeated until a phospholipase coding sequence having an altered codon usage from that of the template nucleic acid is produced.

142. The method of claim 133, wherein the method is iteratively repeated until a phospholipase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

143. A method for modifying codons in a nucleic acid encoding a polypeptide with a phospholipase activity to increase its expression in a host cell, the method comprising the following steps: (a) providing a nucleic acid encoding a polypeptide with a phospholipase activity comprising a sequence as set forth in claim 1 or claim 24; and, (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

144. A method for modifying codons in a nucleic acid encoding a phospholipase polypeptide, the method comprising the following steps: (a) providing a nucleic acid encoding a polypeptide with a phospholipase activity comprising a sequence as set forth in claim 1 or claim 24; and, (b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a phospholipase.

145. A method for modifying codons in a nucleic acid encoding a phospholipase polypeptide to increase its expression in a host cell, the

method comprising the following steps: (a) providing a nucleic acid encoding a phospholipase polypeptide comprising a sequence as set forth in claim 1 or claim 24; and, (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

146. A method for modifying a codon in a nucleic acid encoding a polypeptide having a phospholipase activity to decrease its expression in a host cell, the method comprising the following steps: (a) providing a nucleic acid encoding a phospholipase polypeptide comprising a sequence as set forth in claim 1 or claim 24; and (b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell.

147. The method of claim 146, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.

148. A method for producing a library of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising the following steps: (a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, or a subsequence thereof, and the nucleic acid encodes a phospholipase active site or a phospholipase substrate binding site; (b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, (c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites.

149. The method of claim 148, comprising mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system, gene site-saturation mutagenesis (GSSM), or a synthetic ligation reassembly (SLR).

150. The method of claim 148, comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof.

151. The method of claim 148, comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

152. A method for making a small molecule comprising the following steps: (a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a phospholipase enzyme encoded by a nucleic acid comprising a sequence as set forth in claim 1 or claim 24; (b) providing a substrate for at least one of the enzymes of step (a); and (c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions.

153. A method for modifying a small molecule comprising the following steps: (a) providing a phospholipase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence as set forth in claim 1 or claim 24; (b) providing a small molecule; and (c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the phospholipase enzyme, thereby modifying a small molecule by a phospholipase enzymatic reaction.

154. The method of claim 153, comprising a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the phospholipase enzyme.

155. The method of claim 153, further comprising a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions.

156. The method of claim 155, further comprising the step of testing the library to determine if a particular modified small molecule which exhibits a desired activity is present within the library.

157. The method of claim 156, wherein the step of testing the library further comprises the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

158. A method for determining a functional fragment of a phospholipase enzyme comprising the steps of: (a) providing a phospholipase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a phospholipase activity, thereby determining a functional fragment of a phospholipase enzyme.

159. The method of claim 158, wherein the phospholipase activity is measured by providing a phospholipase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product.

160. A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps: (a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid comprising a sequence as set forth in claim 1 or claim 24; (b) culturing the modified cell to generate a plurality of modified cells; (c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and, (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.

161. The method of claim 160, wherein the genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene.

162. The method of claim 160, further comprising selecting a cell comprising a newly engineered phenotype.

163. The method of claim 162, further comprising culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

164. An isolated or recombinant signal sequence consisting of a sequence as set forth in residues 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30 or 1 to 31, 1 to 32 or 1 to 33 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106.

165. A chimeric polypeptide comprising at least a first domain comprising signal peptide (SP) having a sequence as set forth in claim 164, and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

166. The chimeric polypeptide of claim 165, wherein the heterologous polypeptide or peptide is not a phospholipase.

167. The chimeric polypeptide of claim 165, wherein the heterologous polypeptide or peptide is amino terminal to, carboxy terminal to or on both ends of the signal peptide (SP) or a catalytic domain (CD).

168. An isolated or recombinant nucleic acid encoding a chimeric polypeptide, wherein the chimeric polypeptide comprises at least a first domain comprising signal peptide (SP having a sequence as set forth in claim 164 and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

169. A method of increasing thermotolerance or thermostability of a phospholipase polypeptide, the method comprising **glycosylating** a phospholipase, wherein the polypeptide comprises at least thirty contiguous amino acids of a polypeptide as set forth in claim 61, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, thereby increasing the thermotolerance or thermostability of the phospholipase.

170. A method for overexpressing a recombinant phospholipase in a cell comprising expressing a vector comprising a nucleic acid sequence as set forth in claim 1 or claim 24, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

171. A method of making a transgenic plant comprising the following steps: (a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24, thereby producing a transformed plant cell; (b) producing a transgenic plant from the transformed cell.

172. The method as set forth in claim 171, wherein the step (a) further comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts.

173. The method as set forth in claim 171, wherein the step (a) comprises introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment or by using an Agrobacterium tumefaciens host.

174. A method of expressing a heterologous nucleic acid sequence in a plant cell comprising the following steps: (a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24; (b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell.

175. A method for hydrolyzing, breaking up or disrupting a phospholipid-comprising composition comprising the following steps: (a) providing a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; (b) providing a composition comprising a phospholipid; and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the phospholipase hydrolyzes, breaks up or disrupts the phospholipid-comprising composition.

176. The method as set forth in claim 175, wherein the composition comprises a phospholipid-comprising lipid bilayer or membrane.

177. The method as set forth in claim 175, wherein the composition comprises a plant cell, a bacterial cell, a yeast cell, an insect cell, or an animal cell.

178. A method for liquefying or removing a phospholipid-comprising composition comprising the following steps: (a) providing a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; (b) providing a composition comprising a phospholipid; and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the phospholipase removes or liquefies the phospholipid-comprising composition.

179. A detergent composition comprising a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the polypeptide has a phospholipase activity.

180. The detergent composition of claim 179, wherein the phospholipase is a nonsurface-active phospholipase or a surface-active phospholipase.

181. The detergent composition of claim 179, wherein the phospholipase is formulated in a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel form, a paste or a slurry form.

182. A method for washing an object comprising the following steps: (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; (b) providing an object; and (c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

183. A method for degumming an oil comprising the following steps: (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; (b) providing an composition comprising an phospholipid-containing fat or oil; and (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

184. The method of claim 183, wherein the oil-comprising composition comprises a plant, an animal, an algae or a fish oil or fat.

185. The method of claim 184, wherein plant oil comprises a soybean oil, a rapeseed oil, a corn oil, an oil from a palm kernel, a canola oil, a sunflower oil, a sesame oil or a peanut oil.

186. The method of claim 183, wherein the polypeptide hydrolyzes a phosphatide from a hydratable and/or a non-hydratable phospholipid in the oil-comprising composition.

187. The method of claim 183, wherein the polypeptide hydrolyzes a phosphatide at a glyceryl phosphoester bond to generate a diglyceride and water-soluble phosphate compound.

188. The method of claim 183, wherein the polypeptide has a phospholipase C activity.

189. The method of claim 183, wherein the polypeptide has a phospholipase D activity and a phosphatase enzyme is also added.

190. The method of claim 183, wherein the contacting comprises hydrolysis of a hydrated phospholipid in an oil.

191. The method of claim 183, wherein the hydrolysis conditions of step (c) comprise a temperature of about 20° C. to 40° C. at an alkaline pH.

192. The method of claim 190, wherein the alkaline conditions comprise a pH of about pH 8 to pH 10.

193. The method of claim 183, wherein the hydrolysis conditions of step (c) comprise a reaction time of about 3 to 10 minutes.

194. The method of claim 183, wherein the hydrolysis conditions of step (c) comprise hydrolysis of hydratable and non-hydratable phospholipids in oil at a temperature of about 50° C. to 60° C., at a pH of about pH 5 to pH 6.5 using a reaction time of about 30 to 60 minutes.

195. The method of claim 183, wherein the polypeptide is bound to a filter and the phospholipid-containing fat or oil is passed through the filter.

196. The method of claim 183, wherein the polypeptide is added to a solution comprising the phospholipid-containing fat or oil and then the solution is passed through a filter.

197. A method for converting a non-hydratable phospholipid to a hydratable form comprising the following steps: (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; (b) providing an composition comprising a non-hydratable phospholipid; and (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide converts the non-hydratable phospholipid to a hydratable form.

198. The method of claim 197, wherein the polypeptide has a phospholipase C activity.

199. The method of claim 197, wherein the polypeptide has a phospholipase D activity and a phosphatase enzyme is also added.

200. A method for caustic refining of a phospholipid-containing composition comprising the following steps: (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; (b) providing an composition comprising a phospholipid; and (c) contacting the polypeptide of step (a) with the composition of step (b) before, during or after the caustic refining.

201. The method of claim 200, wherein the polypeptide has a phospholipase C activity.

202. The method of claim 200, wherein the polypeptide having a phospholipase activity is added before caustic refining and the composition comprising the phospholipid comprises a plant and the polypeptide is expressed transgenically in the plant, the polypeptide having a phospholipase activity added during crushing of a seed or other

plant part, or, the polypeptide having a phospholipase activity added following crushing or prior to refining.

203. The method of claim 200, wherein the polypeptide having a phospholipase activity is added during caustic refining and varying levels of acid and caustic are added depending on levels of phosphorous and levels of free fatty acids.

204. The method of claim 200, wherein the polypeptide having a phospholipase activity is added after caustic refining: in an intense mixer or retention mixer prior to separation; following a heating step; in a centrifuge; in a soapstock; in a washwater; or, during bleaching or deodorizing steps.

205. A method for purification of a phytosterol or a triterpene comprising the following steps: (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; (b) providing an composition comprising a phytosterol or a triterpene; and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

206. The method of claim 205, wherein the polypeptide has a phospholipase C activity.

207. The method of claim 205, wherein the phytosterol or a triterpene comprises a plant sterol.

208. The method of claim 207, wherein the plant sterol is derived from a vegetable oil.

209. The method of claim 208, wherein the vegetable oil comprises a coconut oil, canola oil, cocoa butter oil, corn oil, cottonseed oil, linseed oil, olive oil, palm oil, peanut oil, oil derived from a rice bran, safflower oil, sesame oil, soybean oil or a sunflower oil.

210. The method of claim 205, further comprising use of nonpolar solvents to quantitatively extract free phytosterols and phytosteryl fatty-acid esters.

211. The method of claim 205, wherein the phytosterol or a triterpene comprises a β-sitosterol, a campesterol, a stigmasterol, a stigmastanol, a β-sitostanol, a sitostanol, a desmosterol, a chalinasterol, a poriferasterol, a clionasterol or a brassicasterol.

212. A method for refining a crude oil comprising the following steps: (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; (b) providing a composition comprising an oil comprising a phospholipid; and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

213. The method of claim 212, wherein the polypeptide has a phospholipase C activity.

214. The method of claim 212, wherein the polypeptide having a phospholipase activity is in a water solution that is added to the composition.

215. The method of claim 214, wherein the water level is between about 0.5 to 5%.

216. The method of claim 214, wherein the process time is less than about 2 hours.

217. The method of claim 216, wherein the process time is less than about 60 minutes.

218. The method of claim 217, wherein the process time is less than about 30 minutes, less than about 15 minutes, or less than about 5 minutes.

219. The method of claim 212, wherein the hydrolysis conditions comprise a temperature of between about 25° C.-70° C.

220. The method of claim 212, wherein the hydrolysis conditions comprise use of caustics.

221. The method of claim 212, wherein the hydrolysis conditions comprise a pH of between about pH 3 and pH 10.

222. The method of claim 212, wherein the hydrolysis conditions comprise addition of emulsifiers and/or mixing after the contacting of step (c).

223. The method of claim 212, comprising addition of an emulsion-breaker and/or heat to promote separation of an aqueous phase.

224. The method of claim 212, comprising degumming before the contacting step to collect lecithin by centrifugation and then adding a PLC, a PLC and/or a PLA to remove non-hydratable phospholipids.

225. The method of claim 212, comprising water degumming of crude oil to less than 10 ppm for edible oils and subsequent physical refining to less than about 50 ppm for biodiesel oils.

226. The method of claim 212, comprising addition of acid to promote hydration of non-hydratable phospholipids.

227. A method for degumming an oil or a fat comprising the following steps: (a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the phospholipase activity comprises a phospholipase D activity, and a phosphatase enzyme; (b) providing an composition comprising an phospholipid-containing fat or oil; and (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

228. A composition having the equivalent of a phospholipase C activity comprising providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the phospholipase activity comprises a phospholipase D activity, and a phosphatase enzyme.

L13 ANSWER 10 OF 12 USPATFULL on STN
2000:105423 Selectively deglycosylated **human immunodeficiency virus** type 1 **envelope** vaccines.
Essex, Myron E., Sharon, MA, United States

Lee, Tun-Hou, Newton, MA, United States
Lee, Woan-Ruoh, Brookline, MA, United States
Lee, Chun-Nan, Brookline, MA, United States
President and Fellows of Harvard College, Cambridge, MA, United States
(U.S. corporation)
US 6103238 20000815
APPLICATION: US 1992-850770 19920313 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A composition comprising a mutant recombinant **human immunodeficiency virus** type 1 (**HIV**-1) **envelope** glycoprotein which is mutated in its primary amino acid sequence with respect to a wild type **HIV**-1 **envelope** glycoprotein, said mutant glycoprotein including two or more **N-linked** carbohydrate consensus amino acid sequence mutations so as to effect partial deglycosylation, said mutation being positioned between the C terminus of **gp120** and the Cys at the N-terminal side of the **gp120** cysteine loop containing the third hypervariable sequence (V3), said Cys being approximately at amino acid position 296, said mutant glycoprotein being sufficiently deglycosylated such that the total molecular mass of the mutant **gp120** component is less than 75% of the corresponding fully **glycosylated** wild type **gp120** component, said mutant glycoprotein being effective, when present as a component of a complete **HIV** virion, to enable viral infectivity.

2. The mutant glycoprotein composition of claim 1, wherein said virus is **human immunodeficiency virus** type 1, strain selected from the group consisting of MN, HXB2, IIIB, LAI, NL43, MFA, BRVA, SC, JH3, ALAI, BALI, JRCSF, OYI, SF2, NY5CG, SF162, JFL, CDC4, SF33, AN, ADA, WMJ2, RF, ELI, Z2Z6, NDK, JY1, MAL, U455, and Z321.

3. The mutant glycoprotein composition of claim 1, wherein said glycoprotein is **gp160**.

4. The mutant glycoprotein composition of claim 1, wherein said glycoprotein is **gp120**.

5. The mutant glycoprotein composition of claim 1, wherein said primary amino acid sequence is mutated such that one or more consensus **N-linked glycosylation** sequence mutation is a substitution of Asn, Ser, or Thr with a different amino acid.

6. The mutant glycoprotein composition of claim 1 wherein there are deglycosylations at multiple **N-linked glycosylation** attachment sites in the region between the C terminus of **gp120** and the Cys on the N-terminal side of the cysteine loop containing hypervariable region 4 (V4).

7. The mutant glycoprotein composition of claim 1 in which at least one of the **N-linked glycosylation** sequences corresponding to positions 289 and 356 are not mutated.

8. The mutant glycoprotein of claim 1 in which at least one of the **N-linked glycosylation** sequences corresponding to the following position is deglycosylated: 386, 392, 397, 406 and 463.

9. A method of producing antibodies comprising: (a) administering to a mammal a mutant **envelope** protein, said protein being mutated in its primary amino acid sequence with respect to a wild type **HIV**-1 **envelope** glycoprotein, said mutant glycoprotein including two or more **N-linked** carbohydrate consensus amino acid sequence mutations so as

to effect partial deglycosylation, said mutations being positioned between the C terminus of **gp120** and the Cys at the N-terminal side of the **gp120** cysteine loop containing the third hypervariable sequence (V3), said Cys being approximately at amino acid position 296, said mutant glycoprotein being sufficiently deglycosylated such that the total molecular mass of the mutant **gp120** component is less than 75% of the corresponding fully **glycosylated** wild type **gp120** component, said mutant glycoprotein being effective, when present as a component of a complete **HIV** virion, to enable viral infectivity; and (b) recovering said antibodies.

10. The antibodies of claim 9 wherein said antibodies are monoclonal antibodies.

L13 ANSWER 11 OF 12 USPATFULL on STN
1998:162000 Dampening of an immunodominant epitope of an antigen for use in plant, animal and human vaccines and immunotherapies.
Garrity, Robert R., Middletown, MD, United States
Nara, Peter L., Frederick, MD, United States
Goudsmit, Jaap, Amsterdam, Netherlands
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 5853724 19981229
APPLICATION: US 1996-764575 19961213 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An immunogenic composition, comprising: a modified form of a native antigen of a pathogen, the native antigen having disposed at a position thereon an immunodominant epitope comprising a plurality of amino acids, said modified form of the native antigen having a modified epitope at the position of the immunodominant epitope of the native antigen, said modified epitope having been immunodampened so as to substantially redirect an immune response away from the modified epitope and toward a different part of said modified form of the native antigen; and a pharmacologically acceptable carrier.

2. The composition of claim 1, wherein said modified form of the native antigen has a modified amino acid sequence that includes one or more **N-linked glycosylation** signals not present in the native antigen.

3. The composition of claim 1, wherein said carrier comprises a pharmacologically acceptable saline buffer.

4. The composition of claim 1, wherein said modified epitope has been immunodampened by addition of carbohydrate moieties.

5. The composition of claim 1, wherein said modified epitope is immunodampened by an alteration of amino acids.

6. The composition of claim 5, wherein said alteration of amino acids comprises an amino acid substitution.

7. The composition of claim 5, wherein said alteration of amino acids produces an altered set of amino acids against which a humoral response is not substantially produced in a human.

8. The composition of claim 7, wherein said altered set of amino acids comprise a linear human B-cell epitope.

9. The composition of claim 5, wherein said immunodominant epitope has a

native charge and said modified epitope has a modified charge that differs from the native charge of the immunodominant epitope.

10. The composition of claim 5, wherein said alteration comprises deletion of one or more amino acids.

11. The composition of claim 1, wherein said immunodominant epitope includes a binding site for at least one other molecule, and wherein said composition additionally comprises said at least one other molecule irreversibly bound to said immunodominant epitope.

12. The composition of claim 11, wherein said other molecule comprises an antibody directed against said immunodominant epitope.

13. The composition of claim 1, wherein said pathogen is **HIV**-1.

14. The composition of claim 13, wherein said native antigen is **gp120**/160.

L13 ANSWER 12 OF 12 USPATFULL on STN
96:116259 Dampening of an immunodominant epitope of an antigen for use in plant, animal and human compositions and immunotherapies.
Garrity, Robert R., Middletown, MD, United States
Nara, Peter L., Frederick, MD, United States
Goudsmit, Jaap, Amsterdam, Netherlands
The United States of America as represented by the Department of Health Human Services, I
US 5585250 19961217
APPLICATION: US 1993-109934 19930820 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An immunogenic composition comprising; a modified form of **gp120**/160 of **HIV**-1, said modified form of **gp120**/160 having a V3 loop disposed thereon, said V3 loop being immunodampened so as to substantially redirect an immune response away from the V3 loop on the modified form of **gp120**/160 and toward a different part of said modified form of **gp120**/160, and a pharmacologically acceptable carrier.

2. The composition of claim 1, wherein said V3 loop has a modified amino acid sequence that includes one or more **N-linked glycosylation** signals that are not present in native V3 loop.

3. The composition of claim 1, wherein said carrier comprises a pharmacologically acceptable saline buffer.

4. The composition of claim 1, wherein said V3 loop is immunodampened by addition of carbohydrate moieties to said V3 loop.

5. The composition of claim 1, wherein said V3 loop is immunodampened by an alteration of amino acids in said V3 loop.

6. The composition of claim 5, wherein said alteration comprises an amino acid substitution.

7. The composition of claim 6, wherein said alteration of amino acids produces an altered set of amino acids in said V3 loop against which a humoral response is not substantially produced in a human.

8. The composition of claim 7, wherein said altered set of amino acids comprises a linear human B-cell epitope.

9. The composition of claim 5, wherein said V3 loop has a native charge and said alteration results in a change of said native charge.

10. The composition of claim 5, wherein said alteration comprises deletion of one or more amino acids.

11. The composition of claim 1, wherein said V3 loop includes a binding site for at least one other molecule, and wherein said composition additionally comprises said at least one other molecule irreversibly bound to said V3 loop.

12. The composition of claim 11, wherein said other molecule comprises an antibody directed against said V3 loop.

```
=> d l11,exnam,11

L11  ANSWER 11 OF 174  USPATFULL on STN

=> d l13,exnam,11

L13  ANSWER 11 OF 12  USPATFULL on STN
EXNAM  Primary Examiner: Smith, Lynette F.

=> d his

     (FILE 'HOME' ENTERED AT 20:46:21 ON 26 SEP 2006)

     FILE 'USPATFULL' ENTERED AT 20:46:31 ON 26 SEP 2006
                E HAIGWOOD N L/IN
L1            14 S E4

     FILE 'WPIDS' ENTERED AT 20:52:23 ON 26 SEP 2006
                E HAIGWOOD N L/IN
L2            12 S E3

     FILE 'MEDLINE' ENTERED AT 20:54:28 ON 26 SEP 2006
                E HAIGWOOD N L/AU
L3            58 S E3-E5
L4            50 S L3 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L5            35 S L4 AND (ENV? OR GP160 OR GP120 OR GP41)

     FILE 'USPATFULL' ENTERED AT 21:10:23 ON 26 SEP 2006
L6         48156 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN IMMUNOD
L7         32395 S L6 AND (ENV? OR GP160 OR GP120 OR GP41)
L8         12700 S L7 AND GLYCOSYLAT?
L9          4633 S L8 AND (N-LINKED OR O-LINKED)
L10         3810 S L9 AND (NEUTRALIZ?)
L11          174 S L10 AND (ENV?/CLM OR GP160/CLM OR GP120/CLM OR GP41/CLM)
L12           14 S L11 AND (N-LINKED/CLM OR O-LINKED/CLM)
L13           12 S L12 NOT L1

=> file wpids
COST IN U.S. DOLLARS                              SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    39.30     159.48

FILE 'WPIDS' ENTERED AT 21:16:27 ON 26 SEP 2006
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MOST RECENT DERWENT UPDATE:       200661        <200661/DW>
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=> s (HIV or human immunodeficiency virus or SIV or simian immunodeficiency virus)
         22716 HIV
        185772 HUMAN
          8059 IMMUNODEFICIENCY
         44720 VIRUS
          5101 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
           457 SIV
           758 SIMIAN
          8059 IMMUNODEFICIENCY
         44720 VIRUS
           254 SIMIAN IMMUNODEFICIENCY VIRUS
                 (SIMIAN(W)IMMUNODEFICIENCY(W)VIRUS)
L14      23549 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN IMMUNODEFI
               CIENCY VIRUS)

=> s l14 and (env? or gp160 or gp120 or gp41)
        308289 ENV?
           191 GP160
           661 GP120
           339 GP41
L15       2163 L14 AND (ENV? OR GP160 OR GP120 OR GP41)

=> s l15 and glycosylat?
          3336 GLYCOSYLAT?
L16         61 L15 AND GLYCOSYLAT?

=> s l16 and (N-linked or O-linked)
        719325 N
        216755 LINKED
           622 N-LINKED
                 (N(W)LINKED)
        327454 O
        216755 LINKED
           269 O-LINKED
                 (O(W)LINKED)
L17          6 L16 AND (N-LINKED OR O-LINKED)

=> d his

     (FILE 'HOME' ENTERED AT 20:46:21 ON 26 SEP 2006)
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     FILE 'USPATFULL' ENTERED AT 20:46:31 ON 26 SEP 2006
                E HAIGWOOD N L/IN
L1           14 S E4

     FILE 'WPIDS' ENTERED AT 20:52:23 ON 26 SEP 2006
                E HAIGWOOD N L/IN
L2           12 S E3

     FILE 'MEDLINE' ENTERED AT 20:54:28 ON 26 SEP 2006
                E HAIGWOOD N L/AU
L3           58 S E3-E5
L4           50 S L3 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L5           35 S L4 AND (ENV? OR GP160 OR GP120 OR GP41)

     FILE 'USPATFULL' ENTERED AT 21:10:23 ON 26 SEP 2006
L6        48156 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN IMMUNOD
L7        32395 S L6 AND (ENV? OR GP160 OR GP120 OR GP41)
L8        12700 S L7 AND GLYCOSYLAT?
L9         4633 S L8 AND (N-LINKED OR O-LINKED)
L10        3810 S L9 AND (NEUTRALIZ?)
L11         174 S L10 AND (ENV?/CLM OR GP160/CLM OR GP120/CLM OR GP41/CLM)
L12          14 S L11 AND (N-LINKED/CLM OR O-LINKED/CLM)
L13          12 S L12 NOT L1

     FILE 'WPIDS' ENTERED AT 21:16:27 ON 26 SEP 2006
L14       23549 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN IMMUNOD
L15        2163 S L14 AND (ENV? OR GP160 OR GP120 OR GP41)
L16          61 S L15 AND GLYCOSYLAT?
L17           6 S L16 AND (N-LINKED OR O-LINKED)

=> s l17 not l2
L18           4 L17 NOT L2

=> d l18,bib,ab,1-4

L18  ANSWER 1 OF 4  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   2004-480933 [45]   WPIDS
DNC  C2004-178960
TI   Immunogenic mutant HIV-1 gp120 polypeptide, and antibodies raised
     against the polypeptide, useful preventing or treating human
     immunodeficiency virus (HIV) infection, especially HIV-1 infection.
DC   B04 D16
IN   BURTON, D R; PANTOPHLET, R; WILSON, I
PA   (SCRI) SCRIPPS RES INST
CYC  106
PI   WO 2004053100   A2 20040624 (200445)* EN  149
        RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE
            LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH
            PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC
            VN YU ZA ZM ZW
     AU 2003300870   A1 20040630 (200472)
     AU 2003300870   A8 20051103 (200629)
ADT  WO 2004053100 A2 WO 2003-US39534 20031211; AU 2003300870 A1 AU 2003-300870
     20031211; AU 2003300870 A8 AU 2003-300870 20031211
FDT  AU 2003300870 A1 Based on WO 2004053100; AU 2003300870 A8 Based on WO
     2004053100
PRAI US 2003-465350P      20030424; US 2002-432869P      20021211
AB   WO2004053100 A UPAB: 20040716
```

NOVELTY - An immunogenic mutant **HIV gp120** polypeptide that can stimulate a neutralizing antibody response against a **human immunodeficiency virus** (HIV), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for:

(1) an immunogenic mutant **HIV**-1 **gp120** polypeptide (Ia) that can stimulate a neutralizing antibody response against a panel of **human immunodeficiency virus** type 1 (**HIV**-1) comprising **HIV**-1 primary isolates of at least two different clades, where the mutant **gp120** has at least one amino acid mutation in at least one epitope of the **HIV**-1 **gp120** polypeptide specifically bound by a neutralizing antibody, which reduces binding affinity of the non-neutralizing antibody;

(2) an immunogenic mutant **HIV**-1 **gp120** polypeptide (Ib) having at least one amino acid mutation in at least one epitope of the **gp120** polypeptide specifically bound by a non-neutralizing antibody;

(3) a vaccine (II) comprising one or more (Ia);

(4) an antiserum (III) obtained by using (Ib) or (II);

(5) an isolated **HIV** neutralizing antibody (IV) fraction obtained by using (Ib) or (II);

(6) a substantially purified **HIV** neutralizing antibody (V) obtained by using (Ib) or (II);

(7) ameliorating (M1) **HIV**-1 infection in a subject, by administering **HIV** neutralizing produced in response to (Ib);

(8) preventing (M2) **HIV**-1 infection or ameliorating **HIV**-1 infection in a human subject, involves administering **HIV**-1 neutralizing antibodies to the subject, where the **HIV**-1 neutralizing antibodies comprise antibodies stimulated in response to (II);

(9) **HIV** neutralizing antibodies (VI) produced by using (Ib);

(10) isolated **HIV** neutralizing antibodies (VII) obtained by using (Ib); and

(11) isolated **HIV**-1 neutralizing antibodies obtained by harvesting spleen and lymph nodes from the mouse immunized by (II).

ACTIVITY - Anti-**HIV**.

MECHANISM OF ACTION - Vaccine; **HIV** binding agent.

USE - (Ia) and (Ib) are useful for inducing antibodies that can neutralize **HIV**-1 which involves immunizing a subject with a (Ia) or (Ib). The antibodies are useful for preventing or ameliorating **HIV**, especially **HIV**-1, infection in a subject (all claimed).

DESCRIPTION OF DRAWING(S) - The figure shows monoclonal antibody binding wild-type **gp120** and mutant Pro313Asn as determined by enzyme linked immunosorbant assay.

8C, 8D/15

L18 ANSWER 2 OF 4 WPIDS

Full Text

AN 1998-531557 [45] WPIDS
DNC C1998-159424
TI Mutant under-**glycosylated HIV**-1 **envelope** glyco protein composition - useful in, e.g. vaccines to protect mammals, especially humans, against **HIV**-1 infection or to treat such infections.
DC B04 D16
IN DESROSIERS, R C; REITTER, J N
PA (HARD) HARVARD COLLEGE
CYC 21
PI WO 9841536 A1 19980924 (199845)* EN 63
  RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
  W: AU CA JP US
  AU 9865358 A 19981012 (199907)
ADT WO 9841536 A1 WO 1998-US3374 19980313; AU 9865358 A AU 1998-65358 19980313
FDT AU 9865358 A Based on WO 9841536
PRAI US 1997-40790P 19970314
AB WO 9841536 A UPAB: 19981111

A novel composition comprises a recombinant **human immunodeficiency**

virus type 1 (**HIV**-1) **envelope** glycoprotein with an amino acid sequence altered with respect to wild type **HIV**-1 by including a mutated consensus amino acid recognition sequence for **N-linked** carbohydrate attachment. The mutated sequence is positioned on **gp120** between its N-terminus and the Cysteine (at approximately position 296) at the N-terminal side of the **gp120** cysteine hypervariable region 3 (V3) loop. The recombinant **envelope** glycoprotein is not **glycosylated** in mammalian host cells as a result of the mutated sequence ('undergycosylated'), but is effective, when present as a component of a complete **HIV** virion, to support viral infectivity. Also claimed are vaccines comprising the composition to protect humans against **HIV**-1 infection, and antibodies (optionally monoclonal) produced by challenging a mammal with the composition.

USE - The compositions are useful, optionally with suitable carriers, in vaccines (claimed) or pharmaceutical compositions to protect humans against **HIV**-1 infection or treat such infections. Such vaccines may especially comprise mutated **gp120** as above contained in an infective **HIV** virion (claimed). The compositions can also be administered to mammals to induce a protective immune response to prevent/delay **HIV**-1 infection (claimed) or to produce antibodies (claimed). The altered **HIV**-1 **envelope** proteins of the compositions may alternatively be administered by introducing, and allowing expression of, encoding DNA (e.g. using expression vectors). **HIV**-1 **gp120** is heavily **glycosylated** (having approximately 55 % of its molecular mass contributed by **N-linked** carbohydrates) and previous work has demonstrated that, whilst some **N-linked** sites can be eliminated without impairing native structure or ability of the virus to replicate, others (located in the region described above) are essential for the virus to replicate. By selectively removing **N-linked** glycans within this region, an underglycosylated **envelope** glycoprotein capable of enhanced antibody response but retaining infectivity can be produced.

Dwg.0/22

L18 ANSWER 3 OF 4 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 1993-336834 [42] WPIDS

DNC C1993-149019

TI New **glycosylated** immunogenic compsn. contg. **HIV gp120** peptide from V2 region - generates strongly neutralising antibodies useful for treating or preventing infection, in immunoassays, etc..

DC B04 D16

IN PINTER, A; TILLEY, S

PA (PUBL-N) PUBLIC HEALTH RES INST NEW YORK

CYC 19

PI WO 9320104 A1 19931014 (199342)* EN 62
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: AU JP US
AU 9456952 A 19940315 (199428)

ADT WO 9320104 A1 WO 1993-US3044 19930331; AU 9456952 A AU 1994-56952 19930331

FDT AU 9456952 A Based on WO 9320104

PRAI US 1992-860889 19920331

AB WO 9320104 A UPAB: 19940126
Glycosylated immunogenic compsn. contains, apart from a carrier, an **HIV**-gp. 120 peptide (I) which lacks the functional CD4 binding site and the V3 region, but contains at least amino acids 160-180 of gp. 120 of the HXB2 strain (or homologous amino acids of another **HIV**-1 strain). Also new are (1) monoclonal antibodies (MAb) which bind to the specified 160-180 region, but binding to gp. 120 is much reduced by complete removal of **N-linked** carbohydrate residues from the peptide; and (2) compsns. consisting of (a) antibodies whose binding to gp. 120 is competitively inhibited by MAbs to V2 peptides and antibodies specific for the CD-4 binding reigon, these components having synergistic neutralising activity

against **HIV**-1 infectivity in vitro.
Partic., (I) contains the **glycosylated** V2 reigon and can induce prodn. of anti-gp. 120 antibodies having affinity at least 1 x 10 power 9 1/mole.
USE/ADVANTAGE - MAb are strongly neutralising for **HIV**-1 infectivity and form synergistic mixts. with anti-V3 or anti-CD4 binding site antibodies. They can be used to prevent or treat **HIV**-1 infection (e.g., they are admin. to pregnant women to protect the foetus (being of IgG isotype they can cross the placenta) or to persons exposed to **HIV**). They can also be used in immunoassays and for affinity chromatography purificn. (I) are also useful in immunoassays (for detecting **HIV** antigens or antibodies) and as immunogens for antibody prodn. Since MAb are of primate origin, they are stable and only weakly immunogenic when used in humans
Dwg.0/12

L18 ANSWER 4 OF 4 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 1993-303140 [38] WPIDS
DNC C1993-134985
TI Compsn. contg. selectively de-**glycosylated** **HIV**-1 **envelope** protein - shows improved protective immune response.
DC B04 D16
IN ESSEX, M E; LEE, C; LEE, T; LEE, W
PA (HARD) HARVARD COLLEGE
CYC 19
PI WO 9317705 A1 19930916 (199338)* EN 45
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: CA JP
EP 631506 A1 19950104 (199506) EN
R: DE FR GB IT
JP 07504569 W 19950525 (199529)
EP 631506 A4 19970730 (199813)
US 6103238 A 20000815 (200041)
ADT WO 9317705 A1 WO 1993-US1598 19930224; EP 631506 A1 EP 1993-906175 19930224, WO 1993-US1598 19930224; JP 07504569 W JP 1993-515726 19930224, WO 1993-US1598 19930224; EP 631506 A4 EP 1993-906175 19930224; US 6103238 A US 1992-850770 19920313
FDT EP 631506 A1 Based on WO 9317705; JP 07504569 W Based on WO 9317705
PRAI US 1992-850770 19920313
AB WO 9317705 A UPAB: 19931123
Compsn. comprises a mutant recombinant **human immunodeficiency virus** type 1 (**HIV**-1) **envelope** glycoprotein (I), which is mutated in its prim. aminoacid sequence w.r.t a wild type **HIV**-1 **envelope** glycoprotein, but is effective when present as a component of a complete **HIV** virion to enable viral infectivity. (I) includes at least one **N-linked** carbohydrate consensus aminoacid sequence mutation so as to effect partial deglycosylation, the mutation being positioned between the C terminus of gp. 120 and the Cys at the N-terminal side of the gp. 20 cysteine loop contg. the third hypervariable sequence (v3), the Cys being approximately at aminoacid position 296. (I) is sufficiently deglycosylated such that the total mol. mass of the mutant gp 120 component is less than 90% of the corresp. fully **glycosylated** wild type gp. 120 component.
ADVANTAGE - **Glycosylation** serves to reduce or prevent immunological recognition of **envelope** protein domains. Selective deglycosylation enables an immune response to these domains and improves the opportunity for a protective immune response. Thus (I) would be more effective in elicity a protective immune response in people.
Dwg.0/7

=> d his

```
     (FILE 'HOME' ENTERED AT 20:46:21 ON 26 SEP 2006)

     FILE 'USPATFULL' ENTERED AT 20:46:31 ON 26 SEP 2006
                E HAIGWOOD N L/IN
L1           14 S E4

     FILE 'WPIDS' ENTERED AT 20:52:23 ON 26 SEP 2006
                E HAIGWOOD N L/IN
L2           12 S E3

     FILE 'MEDLINE' ENTERED AT 20:54:28 ON 26 SEP 2006
                E HAIGWOOD N L/AU
L3           58 S E3-E5
L4           50 S L3 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L5           35 S L4 AND (ENV? OR GP160 OR GP120 OR GP41)

     FILE 'USPATFULL' ENTERED AT 21:10:23 ON 26 SEP 2006
L6        48156 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN IMMUNOD
L7        32395 S L6 AND (ENV? OR GP160 OR GP120 OR GP41)
L8        12700 S L7 AND GLYCOSYLAT?
L9         4633 S L8 AND (N-LINKED OR O-LINKED)
L10        3810 S L9 AND (NEUTRALIZ?)
L11         174 S L10 AND (ENV?/CLM OR GP160/CLM OR GP120/CLM OR GP41/CLM)
L12          14 S L11 AND (N-LINKED/CLM OR O-LINKED/CLM)
L13          12 S L12 NOT L1

     FILE 'WPIDS' ENTERED AT 21:16:27 ON 26 SEP 2006
L14       23549 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN IMMUNOD
L15        2163 S L14 AND (ENV? OR GP160 OR GP120 OR GP41)
L16          61 S L15 AND GLYCOSYLAT?
L17           6 S L16 AND (N-LINKED OR O-LINKED)
L18           4 S L17 NOT L2

=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      45.32     204.80

FILE 'MEDLINE' ENTERED AT 21:20:35 ON 26 SEP 2006

 FILE LAST UPDATED: 26 Sep 2006 (20060926/UP).  FILE COVERS 1950 TO DATE.

 On December 11, 2005, the 2006 MeSH terms were loaded.

 The MEDLINE reload for 2006 is now (26 Feb.) available.  For details
 on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>).
 See also:

   http://www.nlm.nih.gov/mesh/
   http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
   http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
   http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

 OLDMEDLINE is covered back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2006 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.
```

```
=> s (HIV or human immunodeficiency virus or SIV or simian immunodeficiency virus)
        163138 HIV
       1419595 HUMAN
        124744 IMMUNODEFICIENCY
        419259 VIRUS
         49374 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
          3248 SIV
         20943 SIMIAN
        124744 IMMUNODEFICIENCY
        419259 VIRUS
          4202 SIMIAN IMMUNODEFICIENCY VIRUS
                 (SIMIAN(W)IMMUNODEFICIENCY(W)VIRUS)
L19     169959 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN IMMUNODEFI
               CIENCY VIRUS)

=> s l19 and (env? or gp160 or gp120 or gp41)
        434927 ENV?
          1535 GP160
          6642 GP120
          2627 GP41
L20      15871 L19 AND (ENV? OR GP160 OR GP120 OR GP41)

=> s l20 and glycosylat?
         48330 GLYCOSYLAT?
L21        503 L20 AND GLYCOSYLAT?

=> s l21 and (N-linked or O-linked)
        884633 N
        262882 LINKED
          7022 N-LINKED
                 (N(W)LINKED)
        275923 O
        262882 LINKED
          2789 O-LINKED
                 (O(W)LINKED)
L22        157 L21 AND (N-LINKED OR O-LINKED)

=> s l22 and (neutraliz?)
         53247 NEUTRALIZ?
L23         49 L22 AND (NEUTRALIZ?)

=> d l23,cbib,ab,1-49
```

L23 ANSWER 1 OF 49 MEDLINE on STN
2006548586. PubMed ID: 16973562. **Human Immunodeficiency Virus** Type 1 V1-V2 **Envelope** Loop Sequences Expand and Add **Glycosylation** Sites over the Course of Infection, and These Modifications Affect Antibody **Neutralization** Sensitivity. Sagar Manish; Wu Xueling; Lee Sandra; Overbaugh Julie. (Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N., C3-168, Seattle, WA 98109.. joverbau@fhcrc.org) . Journal of virology, (2006 Oct) Vol. 80, No. 19, pp. 9586-98. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Over the course of infection, **human immunodeficiency virus** type 1 (**HIV**-1) continuously adapts to evade the evolving host **neutralizing** antibody responses. Changes in the **envelope** variable loop sequences, particularly the extent of **glycosylation**, have been implicated in antibody escape. To document modifications that potentially influence antibody susceptibility, we compared **envelope** variable loops 1 and 2 (V1-V2) from multiple sequences isolated at the primary phase of infection to those isolated around 2 to 3 years into the chronic phase of infection in nine women with **HIV**-1 subtype A. **HIV**-1 sequences isolated during

chronic infection had significantly longer V1-V2 loops, with a significantly higher number of potential **N-linked glycosylation** sites, than the sequences isolated early in infection. To assess the effects of these V1-V2 changes on antibody **neutralization** and infectivity, we created chimeric **envelope** sequences, which incorporated a subject's V1-V2 sequences into a common subtype A **envelope** backbone and then used them to generate pseudotyped viruses. Compared to the parent virus, the introduction of a subject's early-infection V1-V2 **envelope** variable loops rendered the chimeric **envelope** more sensitive to that subject's plasma samples but only to plasma samples collected >6 months after the sequences were isolated. **Neutralization** was not detected with the same plasma when the early-infection V1-V2 sequences were replaced with chronic-infection V1-V2 sequences, suggesting that changes in V1-V2 contribute to antibody escape. Pseudotyped viruses with V1-V2 segments from different times in infection, however, showed no significant difference in **neutralization** sensitivity to heterologous pooled plasma, suggesting that viruses with V1-V2 loops from early in infection were not inherently more **neutralization** sensitive. Pseudotyped viruses bearing chimeric **envelopes** with early-infection V1-V2 sequences showed a trend in infecting cells with low CD4 concentrations more efficiently, while engineered viruses with V1-V2 sequences isolated during chronic infection were moderately better at infecting cells with low CCR5 concentrations. These studies suggest that changes within the V1-V2 **envelope** domains over the course of an infection influence sensitivity to autologous **neutralizing** antibodies and may also impact host receptor/coreceptor interactions.

L23 ANSWER 2 OF 49 MEDLINE on STN

2006328821. PubMed ID: 16527321. Immunization with **HIV**-1 SF162-derived **Envelope** gp140 proteins does not protect macaques from heterologous simian-**human immunodeficiency virus** SHIV89.6P infection. Xu Rong; Srivastava Indresh K; Kuller Larene; Zarkikh Irina; Kraft Zane; Fagrouch Zahra; Letvin Norman L; Heeney Jonathan L; Barnett Susan W; Stamatatos Leonidas. (Seattle Biomedical Research Institute, Seattle, WA 98109, USA.) Virology, (2006 Jun 5) Vol. 349, No. 2, pp. 276-89. Electronic Publication: 2006-03-09. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Immunization by the SF162gp140 or the DeltaV2gp140 **HIV-1 envelope** proteins results in the generation of strong homologous **neutralizing** antibodies (NAbs) that offer similar degree of protection from disease-development to macaques challenged with homologous virus. These two immunogens elicit weak cross-reactive NAbs and their effectiveness against heterologous challenge is currently unknown. To examine this issue, we immunized macaques with SIVGag p55 and either the SF162gp140 or the DeltaV2gp140 and challenged them intravenously with SHIV-89.6P. All animals became infected but previous immunization with SF162gp140 accelerated the development of anti-SHIV89.6P **neutralizing** antibody responses following infection. DeltaV2gp140 is derived from SF162gp140 following the deletion of 30 amino acids and one **N-linked glycosylation** site from the V2 loop. Our results suggest that even small differences in **HIV Envelope** immunogen structure can affect the **neutralizing** antibody responses generated following infection.

L23 ANSWER 3 OF 49 MEDLINE on STN

2006234593. PubMed ID: 16641269. **Neutralizing** antibodies do not mediate suppression of **human immunodeficiency virus** type 1 in elite suppressors or selection of plasma virus variants in patients on highly active antiretroviral therapy. Bailey Justin R; Lassen Kara G; Yang Hung-Chih; Quinn Thomas C; Ray Stuart C; Blankson Joel N; Siliciano Robert F. (Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.) Journal of virology, (2006 May) Vol. 80, No. 10, pp. 4758-70. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language:

English.

AB **Neutralizing** antibodies (NAb) against autologous virus can reach high titers in **human immunodeficiency virus** type 1 (**HIV**-1)-infected patients with progressive disease. Less is known about the role of NAb in **HIV**-1-infected patients with viral loads of <50 copies/ml of plasma, including patients on effective highly active antiretroviral therapy (HAART) and elite suppressors, who control **HIV**-1 replication without antiretroviral therapy. In this study, we analyzed full-length **env** sequences from plasma viruses and proviruses in resting CD4(+) T cells of HAART-treated patients, elite suppressors, and untreated **HIV**-1-infected patients with progressive disease. For each patient group, we assessed plasma virus **neutralization** by autologous, contemporaneous plasma. The degree of **env** diversity, the number of **N-linked glycosylation** sites, and the lengths of variable loops were all lower in elite suppressors than in HAART-treated and untreated viremic patients. Both elite suppressors and HAART-treated patients had lower titers of NAb against **HIV**-1 lab strains than those of untreated viremic patients. Surprisingly, titers of NAb against autologous, contemporaneous plasma viruses were similarly low in chronic progressors, elite suppressors, and HAART-treated patients. In elite suppressors and HAART-treated patients, titers of NAb against autologous plasma viruses also did not differ significantly from titers against autologous proviruses from resting CD4(+) T cells. These results suggest that high-titer NAb are not required for maintenance of viral suppression in elite suppressors and that NAb do not select plasma virus variants in most HAART-treated patients. Both drug-mediated and natural suppression of **HIV**-1 replication to levels below 50 copies/ml may limit the stimulation and maintenance of effective NAb responses.

L23 ANSWER 4 OF 49 MEDLINE on STN

2005690562. PubMed ID: 16379001. Consistent patterns of change during the divergence of **human immunodeficiency virus** type 1 **envelope** from that of the inoculated virus in simian/**human immunodeficiency virus**-infected macaques. Blay W M; Gnanakaran S; Foley B; Doria-Rose N A; Korber B T; Haigwood N L. (Department of Pathobiology, University of Washington, Seattle, WA 98195, USA.) Journal of virology, (2006 Jan) Vol. 80, No. 2, pp. 999-1014. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We have analyzed changes to proviral **Env gp120** sequences and the development of **neutralizing** antibodies (NAbs) during 1 year of simian/**human immunodeficiency virus** SHIV-89.6P infection in 11 Macaca nemestrina macaques. Seven macaques had significant **env** divergence from that of the inoculum, and macaques with greater divergence had higher titers of homologous NAbs. Substitutions in sequons encoding potential **N-linked glycosylation** sites (PNGs) were among the first to be established, although overall the total number of sequons did not increase significantly. The majority (19 of 23) of PNGs present in the inoculum were conserved in the sequences from all macaques. Statistically significant variations in PNGs occurred in multiple macaques within constrained regions we term "hot spots," resulting in the selection of sequences more similar to the B consensus. These included additions on V1, the N-terminal side of V4, and the outer region of C2. Complex mutational patterns resulted in convergent PNG shifts in V2 and V5. Charge changes in **Env** V1V2, resulting in a net acidic charge, and a proline addition in V5 occurred in several macaques. Molecular modeling of the 89.6P sequence showed that the conserved glycans lie on the silent face of **Env** and that many are proximal to disulfide bonds, while PNG additions and shifts are proximal to the CD4 binding site. Nonsynonymous-to-synonymous substitution ratios suggest that these changes result from selective pressure. This longitudinal and cross-sectional study of mutations in **human immunodeficiency virus (HIV) env** in the SHIV background provides evidence that there are more constraints on

the configuration of the glycan shield than were previously appreciated.

L23 ANSWER 5 OF 49 MEDLINE on STN
2005489622. PubMed ID: 16160185. Identification of two **N-linked glycosylation** sites within the core of the **simian immunodeficiency virus** glycoprotein whose removal enhances sensitivity to soluble CD4. Pikora Cheryl; Wittish Christine; Desrosiers Ronald C. (New England Primate Research Center, Harvard Medical School, One Pine Hill Drive, Southborough, MA 01722, USA.) Journal of virology, (2005 Oct) Vol. 79, No. 19, pp. 12575-83. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Using PCR mutagenesis to disrupt the NXT/S **N-linked glycosylation** motif of the **Env** protein, we created 27 mutants lacking 1 to 5 of 14 **N-linked glycosylation** sites within regions of **gp120** lying outside of variable loops 1 to 4 within **simian immunodeficiency virus** strain 239 (SIV239). Of 18 mutants missing **N-linked glycosylation** sites predicted to lie within 10 A of CD4 contact sites, the infectivity of 12 was sufficient to measure sensitivity to **neutralization** by soluble CD4 (sCD4), pooled immune sera from SIV239-infected rhesus macaques, and monoclonal antibodies known to **neutralize** certain derivatives of SIV239. Three of these 12 mutants (g3, lacking the 3rd glycan at position 79; g11, lacking the 11th glycan at position 212; and g3,11, lacking both the 3rd and 11th glycans) were approximately five times more sensitive to **neutralization** by sCD4 than wild-type (WT) SIV239. However, these same mutants were no more sensitive to **neutralization** than WT by pooled immune sera. The other 9 of 12 replication-competent mutants in this group were no more sensitive to **neutralization** than the WT by any of the **neutralizing** reagents. Six of the nine mutants that did not replicate appreciably had three or more **glycosylation** sites eliminated; the other three replication-deficient strains involved mutation of site 15. Our results suggest that elimination of glycan attachment sites 3 and 11 enhanced the exposure of contact residues for CD4. Thus, glycans at positions 3 and 11 of SIV239 **gp120** may be particularly important for shielding the CD4-binding site from antibody recognition.

L23 ANSWER 6 OF 49 MEDLINE on STN
2005462342. PubMed ID: 16128920. Infection with a molecularly cloned SIVsm virus elicits high titer homologous **neutralizing** antibodies with heterologous **neutralizing** activity. Mahalanabis M; Hirsch V M; Haigwood N L. (Seattle Biomedical Research Institute, Seattle, WA 98109-5219, USA.) Journal of medical primatology, (2005 Oct) Vol. 34, No. 5-6, pp. 253-61. Journal code: 0320626. ISSN: 0047-2565. Pub. country: Denmark. Language: English.

AB We have evaluated the homologous and heterologous **neutralizing** antibody response in a cohort of six Macaca nemestrina infected with the cloned virus SIVsm62d that showed different levels of **envelope** diversification. Two progressor macaques developed AIDS by 1.5 years post-inoculation and four non-progressors were asymptomatic for 3 years of follow-up. All macaques developed high titers of **neutralizing** antibodies against homologous SIVsm viruses and intermediate titers against SIVsmB670. Heterologous virus **neutralization** of SIVmac, SIVmne, and **HIV**-2 was detected at much lower levels in both progressor macaques; only one of four non-progressors had evidence for broader **neutralizing** antibody activity. We noted changes in potential **N-linked glycosylation** (PNG) sites in V1/V2, C2, and V4 that were common to multiple macaques. These results support a model for viral **neutralization** where heterologous **neutralization** is, in part, driven by a strong homologous response and may be coupled to changes in PNG sites in **envelope**.

L23 ANSWER 7 OF 49 MEDLINE on STN
2005380210. PubMed ID: 16035946. **N-linked glycosylation** in C2 region of **HIV-1 envelope** reduces sensitivity to **neutralizing** antibodies.

Teeraputon Sirilak; Louisirirojchanakul Suda; Auewarakul Prasert. (Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.) Viral immunology, (2005) Vol. 18, No. 2, pp. 343-53. Journal code: 8801552. ISSN: 0882-8245. Pub. country: United States. Language: English.

AB **N-linked glycosylation** at specific sites on **human immunodeficiency virus** (**HIV**)--1 **gp120 envelope** glycoprotein is believed to act as a glycan shield to protect the viral **neutralizing** epitopes. Various **glycosylation** sites have been shown to affect the sensitivity to antibody-mediated **neutralization**. These include sites on V1V2, C2, base of V3, V5 and C5. Among these, the sites around the base of V3 loop have been most consistently found to associate with **neutralization** sensitivity in subtype B viruses. In contrast, we found that **N-linked glycosylation** sites at the junction of V2--C2 and in the middle of C2 were responsible for the **neutralization** resistance in CRF01_A/E, whereas sites at the base of V3 loop and in V1 and V5 did not affect the **neutralization** phenotype.

L23 ANSWER 8 OF 49 MEDLINE on STN

2005325654. PubMed ID: 15975022. **HIV**-1 **envelope** evolution and vaccine efficacy. Mosier D E. (Department of Immunology (IMM7), The Scripps Research Institute, La Jolla, CA 92037, USA.. dmosier@scripps.edu) . Current drug targets. Infectious disorders, (2005 Jun) Vol. 5, No. 2, pp. 171-7. Ref: 123. Journal code: 101128002. ISSN: 1568-0053. Pub. country: Netherlands. Language: English.

AB Transmission of **human immunodeficiency virus** type 1 (**HIV**-1) selects for **envelope** variants with a number of defined properties, including use of CCR5 as the preferred coreceptor, binding to CCR5 in a distinct manner compared to **HIV**-1 isolated later in infection, shorter variable (V) regions, and fewer **N-linked glycosylation** sites. These features define the ideal target for an **envelope**-containing vaccine designed to elicit **neutralizing** antibody. If a candidate vaccine were sufficiently potent to elicit sterilizing immunity, virus evolution would not be an issue. However, all results to date suggest that an **envelope**-containing vaccine will have a lesser impact, and that virus evolution will contribute to escape from the vaccine-induced antibody response. The key question is whether or not the early selection pressure imposed by **neutralizing** antibody will have a long term impact on **HIV** disease progression. Several recent reports suggest that **HIV**-1 will evolve to rapidly escape antibody selection, and that the cost to the virus in terms of entry fitness will be small. Durable effects of vaccination are predicted to be associated with a reduction in peak viremia and viral set point at the time of primary infection.

L23 ANSWER 9 OF 49 MEDLINE on STN

2005251879. PubMed ID: 15890930. The C108g epitope in the V2 domain of **gp120** functions as a potent **neutralization** target when introduced into **envelope** proteins derived from **human immunodeficiency virus** type 1 primary isolates. Pinter Abraham; Honnen William J; D'Agostino Paul; Gorny Miroslaw K; Zolla-Pazner Susan; Kayman Samuel C. (Laboratory of Retroviral Biology, Public Health Research Institute, 225 Warren Street, Newark, NJ 07103-3535, USA.. pinter@phri.org) . Journal of virology, (2005 Jun) Vol. 79, No. 11, pp. 6909-17. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Monoclonal antibodies (MAbs) directed against epitopes in the V2 domain of **human immunodeficiency virus** type 1 **gp120** often possess **neutralizing** activity, but these generally are highly type specific, **neutralize** only laboratory isolates, or have low potency. The most potent of these is C108g, directed against a type-specific epitope in HXB2 and BaL gp120s, which is glycan dependent and, in contrast to previous reports, dependent on intact disulfide bonds. This epitope was introduced into two primary **Envs**, derived from a **neutralization**-sensitive (SF162)

and a **neutralization**-resistant (JR-FL) isolate, by substitution of two residues and, for SF162, addition of an **N-linked glycosylation** site. C108g effectively **neutralized** both variant **Envs** with considerably higher potency than standard MAbs against the V3 and CD4-binding domains and the broadly **neutralizing** MAbs 2G12 and 2F5. These amino acid substitutions also introduced the epitope recognized by a second V2-specific MAb, 10/76b, but this MAb possessed potent **neutralizing** activity only in the absence of the glycan required for C108g reactivity. In contrast to other **gp120**-specific **neutralizing** MAbs, C108g did not block binding of soluble **Env** proteins to either the CD4 or the CCR5 receptor, but studies with a fusion-arrested **Env** indicated that C108g **neutralized** at a step preceding the one blocked by the **gp41**-specific MAb, 2F5. These results indicate that the V1/V2 domain possesses targets that mediate potent **neutralization** of primary viral isolates via a novel mechanism and suggest that inclusion of carbohydrate determinants into these epitopes may help overcome the indirect masking effects that limit the **neutralizing** potency of antibodies commonly produced after infection.

L23 ANSWER 10 OF 49 MEDLINE on STN
2004536680. PubMed ID: 15507649. Evolutionary dynamics of the glycan shield of the **human immunodeficiency virus envelope** during natural infection and implications for exposure of the 2G12 epitope. Dacheux Laurent; Moreau Alain; Ataman-Onal Yasemin; Biron Francois; Verrier Bernard; Barin Francis. (Laboratoire de Virologie, CHU Bretonneau, 37044 Tours cedex, France.) Journal of virology, (2004 Nov) Vol. 78, No. 22, pp. 12625-37. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Elucidation of the kinetics of exposure of **neutralizing** epitopes on the **envelope** of **human immunodeficiency virus** type 1 (**HIV**-1) during the course of infection may provide key information about how **HIV** escapes the immune system or why its **envelope** is such a poor immunogen to induce broadly efficient **neutralizing** antibodies. We analyzed the kinetics of exposure of the epitopes corresponding to the broadly **neutralizing** human monoclonal antibodies immunoglobulin G1b12 (IgG1b12), 2G12, and 2F5 at the quasispecies level during infection. We studied the antigenicity and sequences of 94 full-length **envelope** clones present during primary infection and at least 4 years later in four **HIV**-1 clade B-infected patients. No or only minor exposure differences were observed for the 2F5 and IgG1b12 epitopes between the early and late clones. Conversely, the **envelope** glycoproteins of the **HIV**-1 quasispecies present during primary infection did not expose the 2G12 **neutralizing** epitope, unlike those present after several years in three of the four patients. Sequence analysis revealed major differences at potential **N-linked glycosylation** sites between early and late clones, particularly at positions known to be important for 2G12 binding. Our study, in natural mutants, confirms that the **glycosylation** sites N295, N332, and N392 are essential for 2G12 binding. This study demonstrates the relationship between the evolving "glycan shield " of **HIV** and the kinetics of exposure of the 2G12 epitope during the course of natural infection.

L23 ANSWER 11 OF 49 MEDLINE on STN
2004518898. PubMed ID: 15488604. Mannose binding lectin (MBL) and **HIV**. Ji Xin; Gewurz Henry; Spear Gregory T. (Department of Immunology/Microbiology, Rush-Presbyterian-St. Luke's Medical Center, 1653 W. Congress Pkwy., Chicago, IL 60612, USA.) Molecular immunology, (2005 Feb) Vol. 42, No. 2, pp. 145-52. Ref: 65. Journal code: 7905289. ISSN: 0161-5890. Pub. country: England: United Kingdom. Language: English.

AB The **envelope** protein (**gp120/gp41**) of **HIV**-1 is highly **glycosylated** with about half of the molecular mass of **gp120** consisting of **N-linked** carbohydrates. While **glycosylation** of **HIV** **gp120/gp41** provides a formidable barrier for development of strong

antibody responses to the virus, it also provides a potential site of attack by the innate immune system through the C-type lectin mannose binding lectin (MBL) (also called mannan binding lectin or mannan binding protein). A number of studies have clearly shown that MBL binds to **HIV**. Binding of MBL to **HIV** is dependent on the high-mannose glycans on **gp120** while host cell glycans incorporated into virions do not contribute substantially to this interaction. It is notable that MBL, due to its specificity for the types of glycans that are abundant on **gp120**, has been shown to interact with all tested **HIV** strains. While direct **neutralization** of **HIV** produced in T cell lines by MBL has been reported, **neutralization** is relatively low for **HIV** primary isolates. However, drugs that alter processing of carbohydrates enhance **neutralization** of **HIV** primary isolates by MBL. Complement activation on **gp120** and opsonization of **HIV** due to MBL binding have also been observed but these immune mechanisms have not been studied in detail. MBL has also been shown to block the interaction between **HIV** and DC-SIGN. Clinical studies show that levels of MBL, an acute-phase protein, increase during **HIV** disease. The effects of MBL on **HIV** disease progression and transmission are equivocal with some studies showing positive effects and other showing no effect or negative effects. Because of apparently universal reactivity with **HIV** strains, MBL clearly represents an important mechanism for recognition of **HIV** by the immune system. However, further studies are needed to define the in vivo contribution of MBL to clearance and destruction of **HIV**, the reasons for low **neutralization** by MBL and ways that MBL anti-viral effects can be augmented.

L23 ANSWER 12 OF 49 MEDLINE on STN
2004170964. PubMed ID: 15063126. **Neutralization** sensitivity of a simian-**human immunodeficiency virus** (SHIV-HXBc2P 3.2N) isolated from an infected rhesus macaque with neurological disease. Song Byeongwoon; Cayabyab Mark; Phan Ngoc; Wang Liping; Axthelm Michael K; Letvin Norman L; Sodroski Joseph G. (Department of Cancer Immunology and AIDS, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA.) Virology, (2004 Apr 25) Vol. 322, No. 1, pp. 168-81. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Simian-**human immunodeficiency virus** (SHIV) chimerae, after in vivo passage in monkeys, can induce acquired immunodeficiency syndrome (AIDS)-like illness and death. A monkey infected with the molecularly cloned, pathogenic SHIV-HXBc2P 3.2 exhibited multifocal granulomatous pneumonia as well as progressive neurological impairment characterized by tremors and pelvic limb weakness. SHIV-HXBc2P 3.2N was isolated from brain tissue explants and characterized. Viruses with the **envelope** glycoproteins of SHIV-HXBc2P 3.2N exhibited increased sensitivity to soluble CD4 and several **neutralizing** antibodies compared with viruses with the parental SHIV-HXBc2P 3.2 **envelope** glycoproteins. By contrast, viruses with SHIV-HXBc2P 3.2 and SHIV-HXBc2P 3.2N **envelope** glycoproteins were **neutralized** equivalently by 2G12 and 2F5 antibodies, which are rarely elicited in **HIV**-1-infected humans. A constellation of changes involving both **gp120** and **gp41 envelope** glycoproteins was responsible for the difference in susceptibility to **neutralization** by most antibodies. Surprisingly, the gain of an **N-linked glycosylation** site in the **gp41** ectodomain contributed greatly to **neutralization** sensitivity. Thus, the **environment** of the central nervous system, particularly in the context of immunodeficiency, allows the evolution of immunodeficiency viruses with greater susceptibility to **neutralization** by antibodies.

L23 ANSWER 13 OF 49 MEDLINE on STN
2004141731. PubMed ID: 15033560. The prolonged culture of **human immunodeficiency virus** type 1 in primary lymphocytes increases its sensitivity to **neutralization** by soluble CD4. Pugach Pavel; Kuhmann

Shawn E; Taylor Joann; Marozsan Andre J; Snyder Amy; Ketas Thomas; Wolinsky Steven M; Korber Bette T; Moore John P. (Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, NY 10021, USA.) Virology, (2004 Mar 30) Vol. 321, No. 1, pp. 8-22. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Primary strains of **human immunodeficiency virus** type 1 (**HIV**-1) are known to adapt to replication in cell lines in vitro by becoming sensitive to soluble CD4 (sCD4) and **neutralizing** antibodies (NAb). T-cell lines favor isolation of variants that use CXCR4 as a co-receptor, while primary isolates predominantly use CCR5. We have now studied how a primary R5 isolate, CC1/85, adapts to prolonged replication in primary human peripheral blood mononuclear cells (PBMC). After 19 passages, a variant virus, CCcon.19, had increased sensitivity to both sCD4 and NAb b12 that binds to a CD4-binding site (CD4BS)-associated epitope, but decreased sensitivity to anti-CD4 antibodies. CCcon.19 retains the R5 phenotype, its resistance to other NAbs was unaltered, its sensitivity to various entry inhibitors was unchanged, and its ability to replicate in macrophages was modestly increased. We define CCcon.19 as a primary T-cell adapted (PTCA) variant. Genetic sequence analysis combined with mutagenesis studies on clonal, chimeric viruses derived from CC1/85 and the PTCA variant showed that the most important changes were in the V1/V2 loop structure, one of them involving the loss of an **N-linked glycosylation** site. Monomeric **gp120** proteins expressed from CC1/85 and the PTCA variant did not differ in their affinities for sCD4, suggesting that the structural consequences of the sequence changes were manifested at the level of the native, trimeric **Env** complex. Overall, the adaptation process probably involves selection for variants with higher CD4 affinity and hence greater fusion efficiency, but this also involves the loss of some resistance to **neutralization** by agents directed at or near to the CD4BS. The loss of **neutralization** resistance is of no relevance under in vitro conditions, but NAbs would presumably be a counter-selection pressure against such adaptive changes in vivo, at least when the humoral immune response is intact.

L23 ANSWER 14 OF 49 MEDLINE on STN

2004125675. PubMed ID: 15016849. **N-linked glycosylation** of the V3 loop and the immunologically silent face of **gp120** protects **human immunodeficiency virus** type 1 SF162 from **neutralization** by anti-**gp120** and anti-**gp41** antibodies. McCaffrey Ruth A; Saunders Cheryl; Hensel Mike; Stamatatos Leonidas. (Seattle Biomedical Research Institute. Department of Pathobiology, University of Washington, Seattle, Washington 98109, USA.) Journal of virology, (2004 Apr) Vol. 78, No. 7, pp. 3279-95. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We examined how asparagine-linked glycans within and adjacent to the V3 loop (C2 and C3 regions) and within the immunologically silent face (V4, C4, and V5 regions) of the **human immunodeficiency virus** (**HIV**) SF612 **envelope** affect the viral phenotype. Five of seven potential **glycosylation** sites are utilized when the virus is grown in human peripheral blood mononuclear cells, with the nonutilized sites lying within the V4 loop. Elimination of glycans within and adjacent to the V3 loop renders SF162 more susceptible to **neutralization** by polyclonal **HIV**(+)-positive and simian/**human immunodeficiency virus**-positive sera and by monoclonal antibodies (MAbs) recognizing the V3 loop, the CD4- and CCR5-binding sites, and the extracellular region of **gp41**. Importantly, our studies also indicate that glycans located within the immunologically silent face of **gp120**, specifically the C4 and V5 regions, also conferred on SF162 resistance to **neutralization** by anti-V3 loop, anti-CD4 binding site, and anti-**gp41** MAbs but not by antibodies targeting the coreceptor binding site. We also observed that the amino acid composition of the V4 region contributes to the **neutralization**

phenotype of SF162 by anti-V3 loop and anti-CD4 binding site MAbs. Collectively, our data support the proposal that the **glycosylation** and structure of the immunologically silent face of the **HIV envelope** plays an important role in defining the **neutralization** phenotype of **HIV** type 1.

L23 ANSWER 15 OF 49 MEDLINE on STN
2003589653. PubMed ID: 14671134. Intrapatient alterations in the **human immunodeficiency virus** type 1 **gp120** V1V2 and V3 regions differentially modulate coreceptor usage, virus inhibition by CC/CXC chemokines, soluble CD4, and the b12 and 2G12 monoclonal antibodies. Nabatov Alexey A; Pollakis Georgios; Linnemann Thomas; Kliphius Aletta; Chalaby Moustapha I M; Paxton William A. (Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands.) Journal of virology, (2004 Jan) Vol. 78, No. 1, pp. 524-30. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We studied **human immunodeficiency virus** type 1 (**HIV**-1) chimeric viruses altering in their **gp120** V1V2 and V3 **envelope** regions to better map which genetic alterations are associated with specific virus phenotypes associated with **HIV**-1 disease progression. The V1V2 and V3 regions studied were based on viruses isolated from an individual with progressing **HIV**-1 disease. Higher V3 charges were linked with CXCR4 usage, but only when considered within a specific V1V2 and V3 **N-linked glycosylation** context. When the virus gained R5X4 dual tropism, irrespective of its V3 charge, it became highly resistant to inhibition by RANTES and highly sensitive to inhibition by SDF-1alpha. R5 viruses with higher positive V3 charges were more sensitive to inhibition by RANTES, while R5X4 dualtropic viruses with higher positive V3 charges were more resistant to inhibition by SDF-1alpha. Loss of the V3 **N-linked glycosylation** event rendered the virus more resistant to inhibition by SDF-1alpha. The same alterations in the V1V2 and V3 regions influenced the extent to which the viruses were **neutralized** with soluble CD4, as well as monoclonal antibodies b12 and 2G12, but not monoclonal antibody 2F5. These results further identify a complex set of alterations within the V1V2 and V3 regions of **HIV**-1 that can be selected in the host via alterations of coreceptor usage, CC/CXC chemokine inhibition, CD4 binding, and antibody **neutralization**.

L23 ANSWER 16 OF 49 MEDLINE on STN
2003520646. PubMed ID: 14581570. Genetic and functional analysis of full-length **human immunodeficiency virus** type 1 **env** genes derived from brain and blood of patients with AIDS. Ohagen Asa; Devitt Amy; Kunstman Kevin J; Gorry Paul R; Rose Patrick P; Korber Bette; Taylor Joann; Levy Robert; Murphy Robert L; Wolinsky Steven M; Gabuzda Dana. (Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, Massachusetts, USA.) Journal of virology, (2003 Nov) Vol. 77, No. 22, pp. 12336-45. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The genetic evolution of **human immunodeficiency virus** type 1 (**HIV**-1) in the brain is distinct from that in lymphoid tissues, indicating tissue-specific compartmentalization of the virus. Few primary **HIV**-1 **envelope** glycoproteins (**Envs**) from uncultured brain tissues have been biologically well characterized. In this study, we analyzed 37 full-length **env** genes from uncultured brain biopsy and blood samples from four patients with AIDS. Phylogenetic analysis of intrapatient sequence sets showed distinct clustering of brain relative to blood **env** sequences. However, no brain-specific signature sequence was identified. Furthermore, there was no significant difference in the number or positions of **N-linked glycosylation** sites between brain and blood **env** sequences. The patterns of coreceptor usage were heterogeneous, with no clear distinction between brain and blood **env** clones. Nine **Envs** used CCR5 as a coreceptor, one used CXCR4, and two used both CCR5

and CXCR4 in cell-to-cell fusion assays. Eight **Envs** could also use CCR3, CCR8, GPR15, STRL33, Apj, and/or GPR1, but these coreceptors did not play a major role in virus entry into microglia. Recognition of epitopes by the 2F5, T30, AG10H9, F105, 17b, and C11 monoclonal antibodies varied among **env** clones, reflecting genetic and conformational heterogeneity. **Envs** from two patients contained 28 to 32 N-**glycosylation** sites in **gp120**, compared to around 25 in lab strains and well-characterized primary isolates. These results suggest that **HIV**-1 **Envs** in brain cannot be distinguished from those in blood on the basis of coreceptor usage or the number or positions of N-**glycosylation** sites, indicating that other properties underlie neurotropism. The study also demonstrates characteristics of primary **HIV**-1 **Envs** from uncultured tissues and implies that **Env** variants that are **glycosylated** more extensively than lab strains and well-characterized primary isolates should be considered during development of vaccines and **neutralizing** antibodies.

L23 ANSWER 17 OF 49 MEDLINE on STN
2003507286. PubMed ID: 14557540. **HIV**-1 in genital tract and plasma of women: compartmentalization of viral sequences, coreceptor usage, and **glycosylation**. Kemal Kimdar Sherefa; Foley Brian; Burger Harold; Anastos Kathryn; Minkoff Howard; Kitchen Christina; Philpott Sean M; Gao Wei; Robison Esther; Holman Susan; Dehner Carolyn; Beck Suzanne; Meyer William A 3rd; Landay Alan; Kovacs Andrea; Bremer James; Weiser Barbara. (New York State Department of Health, Wadsworth Center, 120 New Scotland Avenue, Albany, NY 12208, USA.) Proceedings of the National Academy of Sciences of the United States of America, (2003 Oct 28) Vol. 100, No. 22, pp. 12972-7. Electronic Publication: 2003-10-13. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Worldwide, 90% of **HIV**-1 infections are transmitted heterosexually. Because the genital mucosa are the sites of initial contact with **HIV**-1 for most exposed individuals, study of the virus from the genital tract is critical for the development of vaccines and therapeutics. Previous analyses of **HIV**-1 in various tissues have documented compartmentalization of viral genomes. Whether compartmentalization was associated with viral phenotypic differences or immune status, however, was not well understood. We compared **HIV**-1 **gp120 env** sequences from the genital tract and plasma of 12 women. Eight women displayed compartmentalized **HIV**-1 RNA genomes, with viral sequences from each site that were clearly discrete, yet phylogenetically related. The remaining four exhibited **env** sequences that were intermingled between the two sites. Women with compartmentalized **HIV**-1 genomes had higher CD4+ cell counts than those displaying intermingled strains (P = 0.02). Intrapatient **HIV**-1 recombinants comprising sequences that were characteristic of both sites were identified. We next compared viral phenotypes in each compartment. **HIV**-1 coreceptor usage was often compartmentalized (P 0.01). The number of **N-linked glycosylation** sites, associated with **neutralization** resistance, also differed between compartments (P < 0.01). Furthermore, disparities between the density of **gp120 glycosylations** in each compartment correlated with higher CD4+ counts (P = 0.03). These data demonstrate that the genital tract and plasma can harbor populations of replicating **HIV**-1 with different phenotypes. The association of higher CD4+ cell counts with compartmentalization of viral genomes and density of **gp120 glycosylations** suggests that the immune response influences the development of viral genotypes in each compartment. These findings are relevant to the prevention and control of **HIV**-1 infection.

L23 ANSWER 18 OF 49 MEDLINE on STN
2003423729. PubMed ID: 12941910. Assorted mutations in the **envelope** gene of **simian immunodeficiency virus** lead to loss of **neutralization** resistance against antibodies representing a broad spectrum of specificities. Johnson Welkin E; Sanford Hannah; Schwall Linda; Burton

Dennis R; Parren Paul W H I; Robinson James E; Desrosiers Ronald C. (New England Regional Primate Research Center, Department of Microbiology and Molecular Genetics, Harvard Medical School, One Pine Hill Drive, Box 9102, Southborough, MA 01772-9102, USA.) Journal of virology, (2003 Sep) Vol. 77, No. 18, pp. 9993-10003. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Simian immunodeficiency virus (SIV)** of macaques isolate SIVmac239 is highly resistant to **neutralization** by polyclonal antisera or monoclonal antibodies, a property that it shares with most primary isolates of **human immunodeficiency virus** type 1 (**HIV**-1). This resistance is important for the ability of the virus to persist at high levels in vivo. To explore the physical features of the viral **envelope** complex that contribute to the **neutralization**-resistant phenotype, we examined a panel of SIVmac239 derivatives for sensitivity to **neutralization** by a large collection of monoclonal antibodies (MAbs). These MAbs recognize both linear and conformational epitopes throughout the viral **envelope** proteins. The variant viruses included three derivatives of SIVmac239 with substitutions in specific **N-linked glycosylation** sites of **gp120** and a fourth variant that lacked the 100 amino acids that encompass the V1 and V2 loops. Also included in this study was SIVmac316, a variant of SIVmac239 with distributed mutations in **env** that confer significantly increased replicative capacity in tissue macrophages. These viruses were chosen to represent a broad range of **neutralization** sensitivities based on susceptibility to pooled, **SIV**-positive plasma. All three of these very different kinds of mutations (amino acid substitutions, elimination of N-glycan attachment sites, and a 100-amino-acid deletion spanning variable loops V1 and V2) dramatically increased sensitivity to **neutralization** by MAbs from multiple competition groups. Thus, the mutations did not simply expose localized epitopes but rather conferred global increases in **neutralization** sensitivity. The removal of specific N-glycan attachment sites from V1 and V2 led to increased sensitivity to **neutralization** by antibodies recognizing epitopes from both within and outside of the V1-V2 sequence. Surprisingly, while most of the mutations that gave rise to increased sensitivity were located in the N-terminal half of **gp120** (surface subunit [SU]), the greatest increases in sensitivity were to MAbs recognizing the C-terminal half of **gp120** or the ectodomain of **gp41** (transmembrane subunit [TM]). This reagent set and information should now be useful for defining the physical, structural, thermodynamic, and kinetic factors that influence relative sensitivity to antibody-mediated **neutralization**.

L23 ANSWER 19 OF 49 MEDLINE on STN

2003416297. PubMed ID: 12954207. Structure-based, targeted deglycosylation of **HIV-1 gp120** and effects on **neutralization** sensitivity and antibody recognition. Koch Markus; Pancera Marie; Kwong Peter D; Kolchinsky Peter; Grundner Christoph; Wang Liping; Hendrickson Wayne A; Sodroski Joseph; Wyatt Richard. (Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115, USA.) Virology, (2003 Sep 1) Vol. 313, No. 2, pp. 387-400. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The **human immunodeficiency virus (HIV**-1) exterior **envelope** glycoprotein, **gp120**, mediates receptor binding and is the major target for **neutralizing** antibodies. Primary **HIV**-1 isolates are characteristically more resistant to broadly **neutralizing** antibodies, although the structural basis for this resistance remains obscure. Most broadly **neutralizing** antibodies are directed against functionally conserved **gp120** regions involved in binding to either the primary virus receptor, CD4, or the viral coreceptor molecules that normally function as chemokine receptors. These antibodies are known as CD4 binding site (CD4BS) and CD4-induced (CD4i) antibodies, respectively. Inspection of the **gp120** crystal structure reveals that although the receptor-binding

regions lack **glycosylation**, sugar moieties lie proximal to both receptor-binding sites on **gp120** and thus in proximity to both the CD4BS and the CD4i epitopes. In this study, guided by the X-ray crystal structure of **gp120**, we deleted four **N-linked glycosylation** sites that flank the receptor-binding regions. We examined the effects of selected changes on the sensitivity of two prototypic **HIV**-1 primary isolates to **neutralization** by antibodies. Surprisingly, removal of a single **N-linked glycosylation** site at the base of the **gp120** third variable region (V3 loop) increased the sensitivity of the primary viruses to **neutralization** by CD4BS antibodies. **Envelope** glycoprotein oligomers on the cell surface derived from the V3 glycan-deficient virus were better recognized by a CD4BS antibody and a V3 loop antibody than were the wild-type glycoproteins. Absence of all four **glycosylation** sites rendered a primary isolate sensitive to CD4i antibody-mediated **neutralization**. Thus, carbohydrates that flank receptor-binding regions on **gp120** protect primary **HIV**-1 isolates from antibody-mediated **neutralization**.

L23 ANSWER 20 OF 49 MEDLINE on STN
2003132652. PubMed ID: 12646921. Antibody **neutralization** and escape by **HIV**-1. Wei Xiping; Decker Julie M; Wang Shuyi; Hui Huxiong; Kappes John C; Wu Xiaoyun; Salazar-Gonzalez Jesus F; Salazar Maria G; Kilby J Michael; Saag Michael S; Komarova Natalia L; Nowak Martin A; Hahn Beatrice H; Kwong Peter D; Shaw George M. (Howard Hughes Medical Institute, University of Alabama at Birmingham, 720 South 20th Street, KAUL 816, Birmingham, Alabama 35294-0024, USA.) Nature, (2003 Mar 20) Vol. 422, No. 6929, pp. 307-12. Journal code: 0410462. ISSN: 0028-0836. Pub. country: England: United Kingdom. Language: English.

AB **Neutralizing** antibodies (Nab) are a principal component of an effective human immune response to many pathogens, yet their role in **HIV**-1 infection is unclear. To gain a better understanding of this role, we examined plasma from patients with acute **HIV** infection. Here we report the detection of autologous Nab as early as 52 days after detection of **HIV**-specific antibodies. The viral inhibitory activity of Nab resulted in complete replacement of **neutralization**-sensitive virus by successive populations of resistant virus. Escape virus contained mutations in the **env** gene that were unexpectedly sparse, did not map generally to known **neutralization** epitopes, and involved primarily changes in **N-linked glycosylation**. This pattern of escape, and the exceptional density of **HIV**-1 **envelope glycosylation** generally, led us to postulate an evolving 'glycan shield' mechanism of **neutralization** escape whereby selected changes in glycan packing prevent Nab binding but not receptor binding. Direct support for this model was obtained by mutational substitution showing that Nab-selected alterations in **glycosylation** conferred escape from both autologous antibody and epitope-specific monoclonal antibodies. The evolving glycan shield thus represents a new mechanism contributing to **HIV**-1 persistence in the face of an evolving antibody repertoire.

L23 ANSWER 21 OF 49 MEDLINE on STN
2003050181. PubMed ID: 12560567. **Glycosylation** inhibitors and neuraminidase enhance **human immunodeficiency virus** type 1 binding and **neutralization** by mannose-binding lectin. Hart Melanie L; Saifuddin Mohammed; Spear Gregory T. (Department of Immunology/Microbiology, Rush-Presbyterian-St Luke's Medical Center, 1563 W. Congress Parkway, Chicago, IL 60612, USA.) The Journal of general virology, (2003 Feb) Vol. 84, No. Pt 2, pp. 353-60. Journal code: 0077340. ISSN: 0022-1317. Pub. country: England: United Kingdom. Language: English.

AB Mannose-binding lectin (MBL), a C-type lectin component of the human innate immune system, binds to the **gp120 envelope** glycoprotein of **human immunodeficiency virus** type 1 (**HIV-1**). The objective of this study was to assess the effects of inhibitors of endoplasmic

reticulum glucosidases and Golgi mannosidase as well as neuraminidase (NA) on the interaction between **HIV** and MBL. Production of **HIV** in the presence of the mannosidase I inhibitor deoxymannojirimycin (dMM) significantly enhanced binding of **HIV** to MBL and increased MBL **neutralization** of an M-tropic **HIV** primary isolate. In contrast, culturing **HIV** in the presence of alpha-glucosidase I and II inhibitors castanospermine and deoxynojirimycin only slightly affected virus binding and **neutralization** by MBL. Removal of sialic acid from **HIV** by NA also significantly enhanced virus binding and **neutralization** by MBL. Treatment of virus grown in the presence of dMM with endoglycosidase F1 substantially reduced binding to MBL, indicating that dMM increased MBL binding by increasing high-mannose carbohydrates on the virus. In contrast, endoglycosidase F1 did not decrease the MBL interaction with NA-treated virus, suggesting that NA exposed novel MBL binding sites. Treatment with dMM increased the immunocapture of **HIV** by monoclonal antibodies 2F5 and 2G12, indicating that altering the **glycosylation** of viral glycoproteins increases the accessibility or reactivity of some epitopes. This study shows that specific alterations of the **N-linked** carbohydrates on **HIV gp120/gp41** can enhance MBL-mediated **neutralization** of virus by strengthening the interaction of **HIV**-1 with MBL.

L23 ANSWER 22 OF 49 MEDLINE on STN
2002728906. PubMed ID: 12490404. The **N-linked** glycan g15 within the V3 loop of the **HIV**-1 external glycoprotein **gp120** affects coreceptor usage, cellular tropism, and **neutralization**. Polzer Svenja; Dittmar Matthias T; Schmitz Herbert; Schreiber Michael. (Bernhard Nocht Institute for Tropical Medicine, 20359, Hamburg, Germany.) Virology, (2002 Dec 5) Vol. 304, No. 1, pp. 70-80. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB We have studied infectivity and **neutralization** of X4, R5, and R5X4 tropic **HIV**-1 mutants, which are lacking **N-linked glycosylation** sites for glycans g13, g14, g15, and g17 in the V3 loop region of **gp120**. X4-tropic NL4-3 mutants lacking combinations of g14/15 or g15/17 showed markedly higher infectivity in CXCR4-specific infection. The role of g15 in CCR5-specific infection was investigated using viruses with high (NL-918, R5-monotropic), medium (NL-991, R5-monotropic), and low (NL-952, R5X4-dualtropic) CCR5-specific infectivity. For NL-991, a reduction of infectivity on GHOST-CCR5 cells was observed for a mutant lacking g15. For NL-952 mutants all lacking g15, a complete loss of CCR5-specificity was observed and NL-952 was shifted from R5X4 to X4 tropism. For all mutants of NL4-3, NL-991, and NL-952, where the lack of g15 markedly influenced infectivity or coreceptor usage, **neutralization** was enhanced. In contrast, NL-918 mutants with or without g15 showed no difference in **neutralization** and no difference in GHOST-CCR5 infection rates. Thus, for viruses with a low or medium CCR5-specificity the role of g15 for changing CCR5-usage and sensitivity to **neutralization** was more significant than for viruses with high infection rates on GHOST-CCR5 cells. Our data demonstrate that V3 glycans play an important role in the usage of CXCR4 and CCR5. The lack of g15 was relevant for a more efficient use of CXCR4, whereas interaction with CCR5 was facilitated in the presence of g15. This study also demonstrates that glycan g15 is involved in blocking of **neutralizing** antibodies and shifting **HIV** tropism from R5X4 to X4.

L23 ANSWER 23 OF 49 MEDLINE on STN
2002320765. PubMed ID: 12064867. Detection of orientation-specific anti-**gp120** antibodies by a new N-glycanase protection assay. Gram G J; Bolmstedt A; Schonning K; Biller M; Hansen J E S; Olofsson S. (Department of Clinical Virology, University of Goteborg, Sweden.) APMIS : acta pathologica, microbiologica, et immunologica Scandinavica, (2002 Feb) Vol. 110, No. 2, pp. 123-31. Journal code: 8803400. ISSN: 0903-4641. Pub.

country: Denmark. Language: English.

AB Several functions have been assigned to the extensive **glycosylation** of **HIV**-1 **envelope** glycoprotein **gp120**, especially immune escape mechanisms, but the intramolecular interactions between **gp120** and its carbohydrate complement are not well understood. To analyse this phenomenon we established a new microwell deglycosylation assay for determining **N-linked** glycan accessibility after binding of **gp120**-specific agents. Orientation-specific exposition of **gp120** in ELISA microplates was achieved by catching with either anti-C5 antibody D7324 or anti-V3 antibody NEA-9205. We found that soluble CD4 inhibited the deglycosylation of **gp120** only when **gp120** was caught by D7324 and not by NEA9205. In contrast, antibodies from **HIV**-infected individuals inhibited the deglycosylation best when **gp120** was caught by NEA9205. These results demonstrated that both the CD4-binding site and the epitopes recognised by antibodies from **HIV**-infected individuals have N-glycans in the close vicinity. However, the difference in **gp120** orientation indicates that antibodies in **HIV**-infected individuals, at least partly, bind to epitopes different from the CD4-binding site. Finally, we determined the structural class of the glycan of one V1 **glycosylation** site of prototype **HIV**-1 LAI **gp120**, which remained unsolved from previous studies, and found that it belonged to the complex type of glycans.

L23 ANSWER 24 OF 49 MEDLINE on STN

2002199775. PubMed ID: 11932385. Role of **N-linked** glycans in a **human immunodeficiency virus envelope** glycoprotein: effects on protein function and the **neutralizing** antibody response. Quinones-Kochs Miriam I; Buonocore Linda; Rose John K. (Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510, USA.) Journal of virology, (2002 May) Vol. 76, No. 9, pp. 4199-211. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The **envelope** (**Env**) glycoprotein of **human immunodeficiency virus** (**HIV**) contains 24 N-**glycosylation** sites covering much of the protein surface. It has been proposed that one role of these carbohydrates is to form a shield that protects the virus from immune recognition. Strong evidence for such a role for **glycosylation** has been reported for **simian immunodeficiency virus** (**SIV**) mutants lacking glycans in the V1 region of **Env** (J. N. Reitter, R. E. Means, and R. C. Desrosiers, Nat. Med. 4:679-684, 1998). Here we used recombinant vesicular stomatitis viruses (VSVs) expressing **HIV Env glycosylation** mutants to determine if removal of carbohydrates in the V1 and V2 domains affected protein function and the generation of **neutralizing** antibodies in mice. Mutations that eliminated one to six of the sites for **N-linked glycosylation** in the V1 and V2 loops were introduced into a gene encoding the **HIV** type 1 primary isolate 89.6 **envelope** glycoprotein with its cytoplasmic domain replaced by that of the VSV G glycoprotein. The membrane fusion activities of the mutant proteins were studied in a syncytium induction assay. The transport and processing of the mutant proteins were studied with recombinant VSVs expressing mutant **Env** G proteins. We found that **HIV Env** V1 and V2 **glycosylation** mutants were no better than wild-type **envelope** at inducing antibodies **neutralizing** wild-type **Env**, although an **Env** mutant lacking glycans appeared somewhat more sensitive to **neutralization** by antibodies raised to mutant or wild-type **Env**. These results indicate significant differences between **SIV** and **HIV** with regard to the roles of glycans in the V1 and V2 domains.

L23 ANSWER 25 OF 49 MEDLINE on STN

2002131170. PubMed ID: 11861825. CD4 independence of **simian immunodeficiency virus Envs** is associated with macrophage tropism, **neutralization** sensitivity, and attenuated pathogenicity. Puffer Bridget A; Pohlmann Stefan; Edinger Aimee L; Carlin Dan; Sanchez Melissa D;

Reitter Julie; Watry Debbie D; Fox Howard S; Desrosiers Ronald C; Doms Robert W. (Department of Microbiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.) Journal of virology, (2002 Mar) Vol. 76, No. 6, pp. 2595-605. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB To investigate the basis for **envelope** (**Env**) determinants influencing **simian immunodeficiency virus** (**SIV**) tropism, we studied a number of **Envs** that are closely related to that of SIVmac239, a pathogenic, T-tropic virus that is **neutralization** resistant. The **Envs** from macrophage-tropic (M-tropic) virus strains SIVmac316, 1A11, 17E-Fr, and 1100 facilitated infection of CCR5-positive, CD4-negative cells. In contrast, the SIVmac239 **Env** was strictly dependent upon the presence of CD4 for membrane fusion. We also found that the **Envs** from M-tropic virus strains, which are less pathogenic in vivo, were very sensitive to antibody-mediated **neutralization**. Antibodies to the V3-loop, as well as antibodies that block **SIV gp120** binding to CCR5, efficiently **neutralized** CD4-independent, M-tropic **Envs** but not the 239 **Env**. However, triggering the 239 **Env** with soluble CD4, presumably resulting in exposure of the CCR5 binding site, made it as **neutralization** sensitive as the M-tropic **Envs**. In addition, mutations of **N-linked glycosylation** sites in the V1/V2 region, previously shown to enhance antigenicity and immunogenicity, made the 239 **Env** partially CD4 independent. These findings indicate that **Env**-based determinants of M tropism of these strains are generally associated with decreased dependence on CD4 for entry into cells. Furthermore, CD4 independence and M tropism are also associated with **neutralization** sensitivity and reduced pathogenicity, suggesting that the humoral immune response may exert strong selective pressure against CD4-independent M-tropic SIVmac strains. Finally, genetic modification of viral **Envs** to enhance CD4 independence may also result in improved humoral immune responses.

L23 ANSWER 26 OF 49 MEDLINE on STN

2001639963. PubMed ID: 11689624. Conserved, **N-linked** carbohydrates of **human immunodeficiency virus** type 1 **gp41** are largely dispensable for viral replication. Johnson W E; Sauvron J M; Desrosiers R C. (Department of Microbiology and Molecular Genetics, New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772-9102, USA.) Journal of virology, (2001 Dec) Vol. 75, No. 23, pp. 11426-36. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The transmembrane subunit (TM) of **human immunodeficiency virus** type 1 (**HIV**-1) **envelope** protein contains four well-conserved sites for the attachment of **N-linked** carbohydrates. To study the contribution of these N-glycans to the function of TM, we systematically mutated the sites individually and in all combinations and measured the effects of each on viral replication in culture. The mutants were derived from SHIV-KB9, a **simian immunodeficiency virus/HIV** chimera with an **envelope** sequence that originated from a primary **HIV**-1 isolate. The attachment site mutants were generated by replacing the asparagine codon of each N-X-S/T motif with a glutamine codon. The mobilities of the variant transmembrane proteins in sodium dodecyl sulfate-polyacrylamide gel electrophoresis suggested that all four sites are utilized for carbohydrate attachment. Transfection of various cell lines with the resulting panel of mutant viral constructs revealed that the N-glycan attachment sites are largely dispensable for viral replication. Fourteen of the 15 mutants were replication competent, although the kinetics of replication varied depending on the mutant and the cell type. The four single mutants (g1, g2, g3, and g4) and all six double mutants (g12, g13, g14, g23, g24, and g34) replicated in both human and rhesus monkey T-cell lines, as well as in primary rhesus peripheral blood mononuclear cells. Three of the four triple mutants (g124, g134, and g234) replicated in all cell types tested. The triple mutant g123 replicated poorly in

immortalized rhesus monkey T cells (221 cells) and did not replicate detectably in CEMx174 cells. However, at 3 weeks posttransfection of 221 cells, a variant of g123 emerged with a new N-glycan attachment site which compensated for the loss of sites 1, 2, and 3 and resulted in replication kinetics similar to those of the parental virus. The quadruple mutant (g1234) did not replicate in any cell line tested, and the g1234 **envelope** protein was nonfunctional in a quantitative cell-cell fusion assay. The synthesis and processing of the quadruple mutant **envelope** protein appeared similar in transient assays to those of the parental SHIV-KB9 **envelope**. Given their high degree of conservation, the four **N-linked** carbohydrate attachment sites on the external domain of **gp41** are surprisingly dispensable for viral replication. The viral variants described in this report should prove useful for investigation of the contribution of carbohydrate moieties on **gp41** to recognition by antibodies, shielding from antibody-mediated **neutralization**, and structure-function relationships.

L23 ANSWER 27 OF 49 MEDLINE on STN

2001567656. PubMed ID: 11672902. Enhanced immunogenicity of a **human immunodeficiency virus** type 1 **env** DNA vaccine by manipulating N-**glycosylation** signals. Effects of elimination of the V3 N306 glycan. Bolmstedt A; Hinkula J; Rowcliffe E; Biller M; Wahren B; Olofsson S. (Department of Clinical Virology, University of Goteborg, Guldhedsgatan 10 B, S-413 46 Goteborg, Sweden.) Vaccine, (2001 Nov 12) Vol. 20, No. 3-4, pp. 397-405. Journal code: 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.

AB DNA encoding **HIV**-1 **env** is a poorly efficient B-cell immunogen and one probable explanation is that the numerous **gp120 N-linked** glycans **gp120** may interfere with B-cell epitope presentation. The N306 glycan in **gp120** shields **HIV**-1 from **neutralizing** antibodies. A DNA immunogen lacking the N306 **glycosylation** signal (T308A) was constructed to determine whether this glycan affected the immune response. Mice were immunized intranasally twice with DNA containing either the wild type or the mutant **env**. Two additional groups were primed with wild type or mutant **env** and boosted with rgp160 protein, containing the complete set of **N-linked** glycans. Immunization with DNA alone resulted in priming of B-cell clones but was not sufficient to induce a complete antibody response. Animals primed with the N306 mutant and subsequently boosted with rgp160 protein displayed higher serum IgG-binding titers to **gp120** than animals primed with wild type **env** DNA. The manipulation of the **glycosylation** sites of the **env** DNA strongly primes antibody responses (but non-**neutralizing**) as well as T-cell responses to the wild type strain **gp160**. However, priming with mutant plasmid did not result in higher **neutralization** titers to wild type or T308A-mutated virus than did the wild type plasmid. With the N306 mutant DNA we thus immunized a non-**neutralization** epitope, but obtained strong **env**-binding IgG after rgp160 boosting.

L23 ANSWER 28 OF 49 MEDLINE on STN

2001439460. PubMed ID: 11485624. Protection of **neutralization** epitopes in the V3 loop of oligomeric **human immunodeficiency virus** type 1 glycoprotein 120 by **N-linked** oligosaccharides in the V1 region. Losman B; Bolmstedt A; Schonning K; Bjorndal A; Westin C; Fenyo E M; Olofsson S. (Department of Clinical Virology, University of Goteborg, S-413 46 Goteborg, Sweden.) AIDS research and human retroviruses, (2001 Jul 20) Vol. 17, No. 11, pp. 1067-76. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB The V3 region of the **human immunodeficiency virus** type 1 **envelope** protein **gp120** constitutes a potential **neutralization** target, but the oligosaccharide of one conserved N-**glycosylation** site in this region protects it from **neutralizing** antibodies. Here, we determined whether **N-linked** glycans of other **gp120** domains were also involved in

protection of V3 **neutralization** epitopes. Two molecular clones of **HIV**-1, one lacking three **N-linked** glycans of the V1 region (**HIV**-1(3N/V1)) and another lacking three **N-linked** glycans of the C2 region (**HIV**-1(3N/C2)), were created and characterized. **gp120** from both mutated viral clones had higher electrophoretic mobilities than **gp120** from wild-type virus, confirming loss of **N-linked** glycans. Wild-type virus and both mutant clones replicated equally well in established T cell lines and all three viruses were able to utilize CXCR4 but not CCR5 as a coreceptor. The induced mutations increased **gp120** affinity for CXCR4 but caused no corresponding increase in viral ability to replicate in T cell lines. **HIV**-1(3N/V1) was **neutralized** at about 25 times lower concentrations of an antibody to the V3 region than were wild-type virus and **HIV**-1(3N/C2). Soluble, monomeric **gp120** from **HIV**-1(3N/V1) and wild type virus had identical avidity for the V3 antibody, indicating that the V1 glycans were able to shield V3 only in oligomeric but not monomeric **gp120**. In conclusion, one or more **N-linked** glycans of **gp120** V1 is engaged in protection of the V3 region from potential **neutralizing** antibodies, and this effect is dependent on the oligomeric organization of **gp120/gp41**.

L23 ANSWER 29 OF 49 MEDLINE on STN

2001239501. PubMed ID: 11333905. Relationships between CD4 independence, **neutralization** sensitivity, and exposure of a CD4-induced epitope in a **human immunodeficiency virus** type 1 **envelope** protein. Edwards T G; Hoffman T L; Baribaud F; Wyss S; LaBranche C C; Romano J; Adkinson J; Sharron M; Hoxie J A; Doms R W. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.) Journal of virology, (2001 Jun) Vol. 75, No. 11, pp. 5230-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB A CD4-independent version of the X4 **human immunodeficiency virus** type 1 (**HIV-1**) HXBc2 **envelope** (**Env**) protein, termed 8x, mediates infection of CD4-negative, CXCR4-positive cells, binds directly to CXCR4 in the absence of CD4 due to constitutive exposure of a conserved coreceptor binding site in the **gp120** subunit, and is more sensitive to antibody-mediated **neutralization**. To study the relationships between CD4 independence, **neutralization** sensitivity, and exposure of CD4-induced epitopes associated with the coreceptor binding site, we generated a large panel of **Env** mutants and chimeras between 8x and its CD4-dependent parent, HXBc2. We found that a frameshift mutation just proximal to the **gp41** cytoplasmic domain in 8x **Env** was necessary but not sufficient for CD4 independence and led to increased exposure of the coreceptor binding site. In the presence of this altered cytoplasmic domain, single amino acid changes in either the 8x V3 (V320I) or V4/C4 (N386K) regions imparted CD4 independence, with other changes playing a modulatory role. The N386K mutation resulted in loss of an **N-linked glycosylation** site, but additional mutagenesis showed that it was the presence of a lysine rather than loss of the **glycosylation** site that contributed to CD4 independence. However, loss of the **glycosylation** site alone was sufficient to render **Env neutralization** sensitive, providing additional evidence that carbohydrate structures shield important **neutralization** determinants. Exposure of the CD4-induced epitope recognized by monoclonal antibody 17b and which overlaps the coreceptor binding site was highly sensitive to an R298K mutation at the base of the V3 loop and was often but not always associated with CD4 independence. Finally, while not all **neutralization**-sensitive **Envs** were CD4 independent, all CD4-independent **Envs** exhibited enhanced sensitivity to **neutralization** by **HIV**-1-positive human sera, indicating that the humoral immune response can exert strong selective pressure against the CD4-independent phenotype in vivo. Whether this can be used to advantage in designing more effective immunogens remains to be seen.

L23 ANSWER 30 OF 49 MEDLINE on STN
1999292819. PubMed ID: 10364275. Selection for **neutralization** resistance of the simian/**human immunodeficiency virus** SHIVSF33A variant in vivo by virtue of sequence changes in the extracellular **envelope** glycoprotein that modify **N-linked glycosylation**. Cheng-Mayer C; Brown A; Harouse J; Luciw P A; Mayer A J. (Aaron Diamond AIDS Research Center, The Rockefeller University, New York, New York 10016, USA.. cmayer@adarc.org) . Journal of virology, (1999 Jul) Vol. 73, No. 7, pp. 5294-300. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We previously reported on the in vivo adaptation of an infectious molecular simian/**human immunodeficiency virus** (SHIV) clone, SHIVSF33, into a pathogenic biologic viral variant, designated SHIVSF33A. In the present study, we show that SHIVSF33A is resistant to **neutralization** by **human immunodeficiency virus** (**HIV**) and SHIV antisera. Multiple amino acid substitutions accumulated over time throughout the **env** gene of SHIVSF33A; some of them coincided with the acquisition of the **neutralization** resistance of the virus. Of interest are changes that resulted in the removal, repositioning, and addition of potential **glycosylation** sites within the V1, V2, and V3 regions of **envelope gp120**. To determine whether potential **glycosylation** changes within these principal **neutralization** domains of **HIV** type 1 formed the basis for the resistance to serum **neutralization** of SHIVSF33A, mutant viruses were generated on the backbone of parental SHIVSF33 and tested for their **neutralization** sensitivity. The mutations generated did not alter the in vitro replication kinetics or cytopathicity of the mutant viruses in T-cell lines. However, the removal of a potential **glycosylation** site in the V1 domain or the creation of such a site in the V3 domain did allow the virus to escape serum **neutralization** antibodies that recognized parental SHIVSF33. The combination of the V1 and V3 mutations conferred an additive effect on **neutralization** resistance over that of the single mutations. Taken together, these data suggest that (i) SHIV variants with changes in the **Env** SU can be selected in vivo primarily by virtue of their ability to escape **neutralizing** antibody recognition and (ii) carbohydrates play an important role in conferring **neutralization** escape, possibly by altering the structure of **envelope gp120** or by shielding principal **neutralization** sites.

L23 ANSWER 31 OF 49 MEDLINE on STN
1999134336. PubMed ID: 9933605. Probability analysis of variational crystallization and its application to **gp120**, the exterior **envelope** glycoprotein of type 1 **human immunodeficiency virus** (**HIV**-1). Kwong P D; Wyatt R; Desjardins E; Robinson J; Culp J S; Hellmig B D; Sweet R W; Sodroski J; Hendrickson W A. (Department of Biochemistry and Molecular Biophysics, Columbia University, New York, New York 10032, USA.) The Journal of biological chemistry, (1999 Feb 12) Vol. 274, No. 7, pp. 4115-23. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The extensive **glycosylation** and conformational mobility of **gp120**, the **envelope** glycoprotein of type 1 **human immunodeficiency virus** (**HIV**-1), pose formidable barriers for crystallization. To surmount these difficulties, we used probability analysis to determine the most effective crystallization approach and derive equations which show that a strategy, which we term variational crystallization, substantially enhances the overall probability of crystallization for **gp120**. Variational crystallization focuses on protein modification as opposed to crystallization screening. Multiple variants of **gp120** were analyzed with an iterative cycle involving a limited set of crystallization conditions and biochemical feedback on protease sensitivity, **glycosylation** status, and monoclonal antibody binding. Sources of likely conformational heterogeneity such as **N-linked** carbohydrates,

flexible or mobile N and C termini, and variable internal loops were reduced or eliminated, and ligands such as CD4 and antigen-binding fragments (Fabs) of monoclonal antibodies were used to restrict conformational mobility as well as to alter the crystallization surface. Through successive cycles of manipulation involving 18 different variants, we succeeded in growing six different types of **gp120** crystals. One of these, a ternary complex composed of **gp120**, its receptor CD4, and the Fab of the human **neutralizing** monoclonal antibody 17b, diffracts to a minimum Bragg spacing of at least 2.2 A and is suitable for structural analysis.

L23 ANSWER 32 OF 49 MEDLINE on STN
1998285457. PubMed ID: 9623976. A role for carbohydrates in immune evasion in AIDS. Reitter J N; Means R E; Desrosiers R C. (New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772-9102, USA.) Nature medicine, (1998 Jun) Vol. 4, No. 6, pp. 679-84. Journal code: 9502015. ISSN: 1078-8956. Pub. country: United States. Language: English.

AB Rhesus monkeys were infected with mutant forms of **simian immunodeficiency virus** lacking dual combinations of the 4th, 5th and 6th sites for **N-linked glycosylation** in the external **envelope** glycoprotein of the virus. When compared with sera from monkeys infected with the parental virus, sera from monkeys infected with the mutant viruses exhibited markedly increased antibody binding to specific peptides from this region and markedly increased **neutralizing** activity. These results demonstrate a role for **N-linked glycosylation** in limiting the **neutralizing** antibody response to **SIV** and in shielding the virus from immune recognition.

L23 ANSWER 33 OF 49 MEDLINE on STN
1998080428. PubMed ID: 9420239. Evolution of a **simian immunodeficiency virus** pathogen. Edmonson P; Murphey-Corb M; Martin L N; Delahunty C; Heeney J; Kornfeld H; Donahue P R; Learn G H; Hood L; Mullins J I. (Department of Pathology, University of Washington, Seattle 98195-7740, USA.) Journal of virology, (1998 Jan) Vol. 72, No. 1, pp. 405-14. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Analysis of disease induction by simian immunodeficiency viruses (**SIV**) in macaques was initially hampered by a lack of molecularly defined pathogenic strains. The first molecularly cloned **SIV** strains inoculated into macaques, SIVmacBK28 and SIVmacBK44 (hereafter designated BK28 and BK44, respectively), were cases in point, since they failed to induce disease within 1 year postinoculation in any inoculated animal. Here we report the natural history of infection with BK28 and BK44 in inoculated rhesus macaques and efforts to increase the pathogenicity of BK28 through genetic manipulation and in vivo passage. BK44 infection resulted in no disease in four animals infected for more than 7 years, whereas BK28 induced disease in less than half of animals monitored for up to 7 years. Elongation of the BK28 transmembrane protein (TM) coding sequence truncated by prior passage in human cells marginally increased pathogenicity, with two of four animals dying in the third year and one dying in the seventh year of infection. Modification of the BK28 long terminal repeat to include four consensus nuclear factor SP1 and two consensus NF-kappaB binding sites enhanced early virus replication without augmenting pathogenicity. In contrast, in vivo passage of BK28 from the first animal to die from immunodeficiency disease (1.5 years after infection) resulted in a consistently pathogenic strain and a 50% survival time of about 1.3 years, thus corresponding to one of the most pathogenic **SIV** strains identified to date. To determine whether the diverse viral quasispecies that evolved during in vivo passage was required for pathogenicity or whether a more virulent virus variant had evolved, we generated a molecular clone composed of the 3' half of the viral genome

derived from the in vivo-passaged virus (H824) fused with the 5' half of the BK28 genome. Kinetics of disease induction with this cloned virus (BK28/H824) were similar to those with the in vivo-passaged virus, with four of five animals surviving less than 1.7 years. Thus, evolution of variants with enhanced pathogenicity can account for the increased pathogenicity of this **SIV** strain. The genetic changes responsible for this virulent transformation included at most 59 point mutations and 3 length-change mutations. The critical mutations were likely to have been multiple and dispersed, including elongation of the TM and Nef coding sequences; changes in RNA splice donor and acceptor sites, TATA box sites, and Sp1 sites; multiple changes in the V2 region of SU, including a consensus **neutralization** epitope; and five new **N-linked glycosylation** sites in SU.

L23 ANSWER 34 OF 49 MEDLINE on STN

97456542. PubMed ID: 9311856. Specific **N-linked** and **O-linked glycosylation** modifications in the **envelope** V1 domain of **simian immunodeficiency virus** variants that evolve in the host alter recognition by **neutralizing** antibodies. Chackerian B; Rudensey L M; Overbaugh J. (Department of Microbiology, University of Washington, Seattle 98195, USA.) Journal of virology, (1997 Oct) Vol. 71, No. 10, pp. 7719-27. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB During progression to AIDS in **simian immunodeficiency virus (SIV)** Mne-infected macaques, viral variants are selected that encode sequences with serine and threonine changes in variable region 1 (V1) of the surface component of the viral **envelope** protein (**Env**-SU). Because these serine and threonine amino acid changes are characteristic of sites for **O-linked** and **N-linked glycosylation**, we examined whether they were targets for modification by carbohydrates. For this purpose, we used several biochemical methods for analyzing the **Env**-SU protein encoded by chimeras of SIVMneCL8 and **envelope** sequences cloned from an SIVMneCL8-infected Macaca nemestrina during clinical latency and just after the onset of AIDS. The addition of an **N-linked** glycan was demonstrated by changes in the electrophoretic mobility of **Env**-SU, and this was verified by specific glycanase digestions and a detailed analysis of the molecular mass of partially purified **Env**-SU by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Molecular mass calculations by MALDI-TOF MS also demonstrated an increased mass, from 102.3 to 103.5 kDa, associated with serine and threonine residues predicted to be **O-linked glycosylation** sites. Together, these data provide the first direct evidence that the carbohydrate profile of **Env**-SU is distinct in **SIV** variants that evolve during infection of the host. Moreover, our studies show that these changes in **glycosylation** in V1 were directly associated with changes in antigenicity. Specifically, serine and threonine changes in V1 allowed the virus to escape **neutralization** by macaque sera that contained antibodies that could **neutralize** the parental virus, SIVMneCL8. The escape from antibody recognition appeared to be influenced by either **O-linked** or **N-linked** carbohydrate additions in V1. Moreover, when glycine residues were engineered at the positions where serine and threonine changes evolve in V1 of SIVMneCL8, there was no change in antigenicity compared to SIVMneCL8. This suggests that the amino acids in V1 are not part of the linear epitope recognized by **neutralizing** antibody. More likely, V1-associated carbohydrates mask the major **neutralizing** epitope of **SIV**. These experiments indicate that the selection of novel **glycosylation** sites in the V1 region of **envelope** during the course of disease is driven by humoral immune responses.

L23 ANSWER 35 OF 49 MEDLINE on STN

97454133. PubMed ID: 9312273. **Glycosylation** affects both the three-dimensional structure and antibody binding properties of the

HIV-1IIIB **GP120** peptide RP135. Huang X; Barchi J J Jr; Lung F D; Roller P P; Nara P L; Muschik J; Garrity R R. (Laboratory of Medicinal Chemistry, Division of Basic Sciences, National Cancer Institute, Bethesda, Maryland 20892, USA.) Biochemistry, (1997 Sep 9) Vol. 36, No. 36, pp. 10846-56. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB We have prepared **glycosylated** analogues of the principal **neutralizing** determinant of **gp120** and studied their conformations by NMR and circular dichroism spectroscopies. The 24-residue peptide from the **HIV**-1IIIB isolate (residues 308-331) designated RP135, which contains the immunodominant tip of the V3 loop, was **glycosylated** with both N- and **O-linked** sugars. The structures of two glycopeptides, one with an **N-linked** beta-glucosamine (RP135NG) and the other with two **O-linked** alpha-galactosamine units (RP135digal), were studied by NMR and circular dichroism spectroscopies. Molecular dynamics calculations based on the NMR data obtained in water solutions were performed to explore the conformational substates sampled by the glycopeptides. The data showed that covalently linking a carbohydrate to the peptide has a major effect on the local conformation and imparts additional minor changes at more distant sites of partially defined secondary structure. In particular, the transient beta-type turn comprised of the -Gly-Pro-Gly-Arg- segment at the "tip" of the V3 loop is more highly populated in RP135digal that in the native peptide and **N-linked** analogue. Binding data for the glycopeptides with 0.5beta, a monoclonal antibody mapped to the RP135 sequence, revealed a significant enhancement in binding for RP135digal as compared with the native peptide, whereas binding was reduced for the **N-linked** glycopeptide. These data show that **glycosylation** of V3 loop peptides can affect their conformations as well as their interactions with antibodies. The design of more ordered and biologically relevant conformations of immunogenic regions from **gp120** may aid in the design of more effective immunogens for **HIV**-1 vaccine development.

L23 ANSWER 36 OF 49 MEDLINE on STN

97343937. PubMed ID: 9200464. Refocusing **neutralizing** antibody response by targeted dampening of an immunodominant epitope. Garrity R R; Rimmelzwaan G; Minassian A; Tsai W P; Lin G; de Jong J J; Goudsmit J; Nara P L. (Division of Basic Sciences, National Cancer Institute-Frederick Cancer Research and Development Center (NCI-FCRDC), MD 21702, USA.. garrity@sri.org) . Journal of immunology (Baltimore, Md. : 1950), (1997 Jul 1) Vol. 159, No. 1, pp. 279-89. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Immunodominant epitopes are known to suppress a primary immune response to other antigenic determinants by a number of mechanisms. Many pathogens have used this strategy to subvert the immune response and may be a mechanism responsible for limited vaccine efficacies. **HIV**-1 vaccine efficacy appears to be complicated similarly by a limited, immunodominant, isolate-restricted immune response generally directed toward determinants in the third variable domain (V3) of the major **envelope** glycoprotein, **gp120**. To overcome this problem, we have investigated an approach based on masking the V3 domain through addition of **N-linked** carbohydrate and reduction in net positive charge. **N-linked** modified gp120s were expressed by recombinant vaccinia virus and used to immunize guinea pigs by infection and protein boosting. This modification resulted in variable site-specific **glycosylation** and antigenic dampening, without loss of **gp120**/CD4 binding or virus **neutralization**. Most importantly, V3 epitope dampening shifted the dominant type-specific **neutralizing** Ab response away from V3 to an epitope in the first variable domain (V1) of **gp120**. Interestingly, in the presence of V3 dampening V1 changes from an immunodominant non-**neutralizing** epitope to a primary **neutralizing** epitope with broader **neutralizing** properties. In addition, Ab responses were also observed to conserved domains in C1 and C5. These results

suggest that selective epitope dampening can lead to qualitative shifts in the immune response resulting in second order **neutralizing** responses that may prove useful in the fine manipulation of the immune response and in the development of more broadly protective vaccines and therapeutic strategies.

L23 ANSWER 37 OF 49 MEDLINE on STN
96275629. PubMed ID: 8673525. Influence of **N-linked** glycans in V4-V5 region of **human immunodeficiency virus** type 1 glycoprotein **gp160** on induction of a virus-**neutralizing** humoral response. Bolmstedt A; Sjolander S; Hansen J E; Akerblom L; Hemming A; Hu S L; Morein B; Olofsson S. (Department of Clinical Virology, University of Goteborg, Sweden.) Journal of acquired immune deficiency syndromes and human retrovirology : official publication of the International Retrovirology Association, (1996 Jul) Vol. 12, No. 3, pp. 213-20. Journal code: 9501482. ISSN: 1077-9450. Pub. country: United States. Language: English.

AB One of the functions of **N-linked** glycans of viral glycoproteins is protecting otherwise accessible **neutralization** epitopes of the viral **envelope** from **neutralizing** antibodies. The aim of the present study was to explore the possibility to obtain a more broadly **neutralizing** immune response by immunizing guinea pigs with **gp160** depleted of three **N-linked** glycans in the CD4-binding domain by site-directed mutagenesis. Mutant and wild type **gp160** were formulated into immunostimulating complexes and injected s.c. into guinea pigs. Both preparations induced high serum antibody response to native **gp120** and V3 peptides. Both preparations also induced antibodies that bound equally well to the V3 loop or the CD4-binding region, as determined by a competitive enzyme-linked immunosorbent assay (ELISA). The sera from animals, immunized with mutated glycoprotein, did not **neutralize** nonrelated **HIV** strains better than did sera from animals, immunized with wild type glycoprotein. Instead, a pattern of preferred homologous **neutralization** was observed, i.e., sera from animals, immunized with mutant **gp160**, **neutralized** mutant virus better than wild type virus, and vice versa. These data indicated that elimination of the three **N-linked** glycans from **gp160** resulted in an altered local antigenic conformation but did not uncover hidden **neutralization** epitopes, broadening the immune response.

L23 ANSWER 38 OF 49 MEDLINE on STN
96266411. PubMed ID: 8661410. Antigenic variation of **SIV**: mutations in V4 alter the **neutralization** profile. Kinsey N E; Anderson M G; Unangst T J; Joag S V; Narayan O; Zink M C; Clements J E. (Department of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.) Virology, (1996 Jul 1) Vol. 221, No. 1, pp. 14-21. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Antigenic variation is a characteristic feature of lentiviral infection. The **SIV**/macaque model of AIDS provides an ideal system in which to investigate the molecular basis of antigenic variation. The purpose of this study was to genetically map the nucleotide changes in **env** that alter the **neutralization** phenotype of **SIV**. Serum taken from an SIVmac239-infected macaque (2D) at 30 weeks postinoculation was found to **neutralize** the input virus (SIVmac239) and an isolate, P9, obtained at 10 weeks p.i., but did not **neutralize** two other isolates, P13 and P23, obtained at 20 and 52 weeks, respectively. Sequence analysis of these virus variants revealed clustered amino acid changes in V1 and single base pair changes in V2-V4 of P13 and P23. Infectious recombinant viruses in which the V1 and V1-V3 sequences of SIVmac239 were replaced with those of P13 or P23 retained the **neutralization** profile of SIVmac239; both were **neutralized** by macaque 2D serum. Recombinants containing the entire surface glycoprotein (**gp120**) (V1-V5) and the 5' portion of **gp41** of P13 and P23 and those containing **gp120** sequences from V4 through the 5'

portion of the transmembrane glycoprotein (**gp41**) were not **neutralized** by 2D serum. Using a panel of monoclonal antibodies in radioimmunoprecipitation assays, P23 and recombinants containing V4 and V5 of P23 were shown to be antigenically distinct from P13 and SIVmac239. The majority of the amino acid changes in the antigenically distinct viruses were clustered in V4 (amino acids 413-418) and these changes created new potential **N-linked glycosylation** sites. This study demonstrates that a small number of specific amino acid changes (amino acids 412 to 418 in the **env** gene) in the V4 region of the **SIV envelope** glycoprotein can alter antibody recognition and **neutralization** and that these phenotypic changes may be associated with altered **glycosylation** of the **envelope**.

L23 ANSWER 39 OF 49 MEDLINE on STN
96203963. PubMed ID: 8627264. Rapid selection for an **N-linked** oligosaccharide by monoclonal antibodies directed against the V3 loop of **human immunodeficiency virus** type 1. Schonning K; Jansson B; Olofsson S; Hansen J E. (Department 144, Hvidovre Hospital, Denmark.) The Journal of general virology, (1996 Apr) Vol. 77 (Pt 4), pp. 753-8. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The V3 loop of the **human immunodeficiency virus** (**HIV**) surface protein, gp 120, constitutes a principal **neutralizing** determinant. **HIV** strains lacking a naturally conserved **N-linked** oligosaccharide (at position 306) within the V3 loop are highly sensitive to **neutralization**. We subjected molecular clones of **HIV**(LAI) lacking this 306N-glycan to in vitro immune selection with MAbs directed against the V3 loop. In all, ten clones were characterized, and all proved resistant to V3-directed **neutralization**. Sequencing of the V3 loop revealed that six of the clones had become resistant at least partly by reacquisition of the 306N-glycan. Only two of the clones possessed mutations within the binding site of the antibody itself, while the two remaining clones did not display changes within the V3 loop itself. Thus, **HIV** strains lacking the 306N-glycan primarily develop resistance to V3-directed **neutralization** through acquisition of the specific oligosaccharide. This demonstrates that protein **glycosylation** can be a primary modifier of virus antigenicity of possible importance for the interaction of **HIV** with the host immune response.

L23 ANSWER 40 OF 49 MEDLINE on STN
96195257. PubMed ID: 8634021. Sensitivity of **HIV**-1 to **neutralization** by antibodies against **O-linked** carbohydrate epitopes despite deletion of O-**glycosylation** signals in the V3 loop. Hansen J E; Jansson B; Gram G J; Clausen H; Nielsen J O; Olofsson S. (Laboratory for Infectious Diseases, Hvidovre Hospital, Hvidovre, Denmark.) Archives of virology, (1996) Vol. 141, No. 2, pp. 291-300. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB It has been suggested that threonine or serine residues in the V3 loop of **HIV**-1 **gp120** are **glycosylated** with the short-chain **O-linked** oligosaccharides Tn or sialosyl-Tn that function as epitopes for broadly **neutralizing** carbohydrate specific antibodies. In this study we examined whether mutation of such threonine or serine residues could decrease the sensitivity to infectivity inhibition by Tn or sialosyl-Tn specific antibodies. All potentially O-**glycosylated** threonine and serine residues in the V3 loop of cloned **HIV**-1 BRU were mutagenized to alanine thus abrogating any O-**glycosylation** at these sites. Additionally, one of these T-A mutants (T308A) also abrogated the signal for N-**glycosylation** at N306 inside the V3-loop. The mutant clones were compared with the wild type virus as to sensitivity to **neutralization** with monoclonal and polyclonal antibodies specific for the tip of the V3 loop of BRU or for the **O-linked** oligosaccharides Tn or sialosyl-Tn. Deletion of the **N-linked** oligosaccharide at N306 increased the

neutralization sensitivity to antibodies specific for the tip of the loop, which indicates that **N-linked glycosylation** modulates the accessibility to this immunodominant epitope. However, none of the mutants with deletions of O-**glycosylation** signals in the V3 loop displayed any decrease in sensitivity to anti-Tn or anti-sialosyl-Tn antibody. This indicates that these broadly specific **neutralization** epitopes are located outside the V3 loop of gp 120.

L23 ANSWER 41 OF 49 MEDLINE on STN
96135224. PubMed ID: 8551569. Human monoclonal antibody 2G12 defines a distinctive **neutralization** epitope on the **gp120** glycoprotein of **human immunodeficiency virus** type 1. Trkola A; Purtscher M; Muster T; Ballaun C; Buchacher A; Sullivan N; Srinivasan K; Sodroski J; Moore J P; Katinger H. (Aaron Diamond AIDS Research Center, New York University School of Medicine, New York 10016, USA.) Journal of virology, (1996 Feb) Vol. 70, No. 2, pp. 1100-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We have isolated and characterized human monoclonal antibody 2G12 to the **gp120** surface glycoprotein of **human immunodeficiency virus** type 1 (**HIV**-1). This antibody potently and broadly **neutralizes** primary and T-cell line-adapted clade B strains of **HIV**-1 in a peripheral blood mononuclear cell-based assay and inhibits syncytium formation in the AA-2 cell line. Furthermore, 2G12 possesses **neutralizing** activity against strains from clade A but not from clade E. Complement- and antibody-dependent cellular cytotoxicity-activating functions of 2G12 were also defined. The **gp120** epitope recognized by 2G12 was found to be distinctive; binding of 2G12 to LAI recombinant **gp120** was abolished by amino acid substitutions removing **N-linked** carbohydrates in the C2, C3, V4, and C4 regions of **gp120**. This **gp120** mutant recognition pattern has not previously been observed, indicating that the 2G12 epitope is unusual. consistent with this, antibodies able to block 2G12 binding to recombinant **gp120** were not detected in significant quantities in 16 **HIV**-positive human serum samples.

L23 ANSWER 42 OF 49 MEDLINE on STN
95134083. PubMed ID: 7832633. Identification of an **N-linked** glycan in the V1-loop of **HIV**-1 **gp120** influencing **neutralization** by anti-V3 antibodies and soluble CD4. Gram G J; Hemming A; Bolmstedt A; Jansson B; Olofsson S; Akerblom L; Nielsen J O; Hansen J E. (Laboratory for Infectious Diseases, Hvidovre Hospital, Denmark.) Archives of virology, (1994) Vol. 139, No. 3-4, pp. 253-61. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB **Glycosylation** is necessary for **HIV**-1 **gp120** to attain a functional conformation, and individual **N-linked** glycans of **gp120** are important, but not essential, for replication of **HIV**-1 in cell culture. We have constructed a mutant **HIV**-1 infectious clone lacking a signal for **N-linked glycosylation** in the V1-loop of **HIV**-1 **gp120**. Lack of an **N-linked** glycan was verified by a mobility enhancement of mutant **gp120** in SDS-gel electrophoresis. The mutated virus showed no differences in either **gp120** content per infectious unit or infectivity, indicating that the **N-linked** glycan was neither essential nor affecting viral infectivity in cell culture. We found that the mutated virus lacking an **N-linked** glycan in the V1-loop of **gp120** was more resistant to **neutralization** by monoclonal antibodies to the V3-loop and **neutralization** by soluble recombinant CD4 (sCD4). Both viruses were equally well **neutralized** by ConA and a conformation dependent human antibody IAM-2G12. This suggests that the **N-linked** glycan in the V1-loop modulates the three-dimensional conformation of **gp120**, without changing the overall functional integrity of the molecule.

L23 ANSWER 43 OF 49 MEDLINE on STN
95018674. PubMed ID: 7933144. Insertion of **N-linked glycosylation**

sites in the variable regions of the **human immunodeficiency virus** type 1 surface glycoprotein through AAT triplet reiteration. Bosch M L; Andeweg A C; Schipper R; Kenter M. (Laboratory of Immunobiology, National Institute for Public Health and Environmental Protection, The Netherlands.) Journal of virology, (1994 Nov) Vol. 68, No. 11, pp. 7566-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Variable regions with sequence length variation in the **human immunodeficiency virus** type 1 **envelope** exhibit an unusual pattern of codon usage with AAT, ACT, and AGT together composing > 70% of all codons used. We postulate that this distribution is caused by insertion of AAT triplets followed by point mutations and selection. Accumulation of the encoded amino acids (asparagine, serine, and threonine) leads to the creation of new **N-linked glycosylation** sites, which helps the virus to escape from the immune pressure exerted by virus-**neutralizing** antibodies.

L23 ANSWER 44 OF 49 MEDLINE on STN

94267927. PubMed ID: 7515975. A novel, glycan-dependent epitope in the V2 domain of **human immunodeficiency virus** type 1 **gp120** is recognized by a highly potent, **neutralizing** chimpanzee monoclonal antibody. Warrier S V; Pinter A; Honnen W J; Girard M; Muchmore E; Tilley S A. (Public Health Research Institute, New York, New York 10016.) Journal of virology, (1994 Jul) Vol. 68, No. 7, pp. 4636-42. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB An anti-**gp120** monoclonal antibody (MAb), C108G (gamma 1, kappa), was isolated from a chimpanzee that had been infected with strain IIIB of **human immunodeficiency virus** type 1 (**HIV**-1IIIB) and subsequently immunized with the recombinant glycoprotein rgp160MN. This MAb is specific for the IIIB strain of **HIV**-1 and related clones and exhibits very potent **neutralization** of these viruses; e.g., 100% **neutralization** of approximately 8 x 10(3) infectious units of HXB2 was achieved with 125 ng of C108G per ml. Commensurate with this potent **neutralizing** activity, the apparent affinity of C108G for rgp160LAI was very high, i.e., approximately 3 x 10(10) liters/mol. The C108G epitope was not destroyed by reduction of **gp120** disulfide bonds but was profoundly disrupted by removal of **N-linked** sugars from **gp120**. Despite the importance of a glycan(s) in forming the C108G epitope, specific binding of C108G to synthetic peptides overlapping in amino acids 162 to 169 of the V2 region was detected, albeit with an affinity approximately 2,000-fold lower than that of C108G's binding to **glycosylated envelope** protein. This epitope mapping correlated with results of competition assays using MAbs of known epitope specificities. To our knowledge, this is the first description of an anti-V2 MAb raised in response to **HIV**-1 infection. Its potent **neutralizing** activity and epitope specificity indicate that the V2 domain of **gp120** may be an effective target of the protective immune response and, therefore, potentially an important component of **HIV** vaccines.

L23 ANSWER 45 OF 49 MEDLINE on STN

94030750. PubMed ID: 8216976. Diversity of the V3 region of **HIV** in Paris, France. Chaix M L; Chappey C; Couillin I; Rozenbaum W; Levy J P; Saragosti S. (ICGM, INSERM U363, Hopital Cochin, Paris, France.) AIDS (London, England), (1993 Sep) Vol. 7, No. 9, pp. 1199-204. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

AB OBJECTIVE: To carry out, within France, a large-scale molecular epidemiological investigation on the principal **neutralizing** determinant of **HIV**-1, located in the third variable region (V3) of the **envelope** protein. Such investigations are of the utmost importance in the identification and monitoring of the distribution and spread of different viral strains internationally. DESIGN: Using polymerase chain reaction (PCR), we examined the genetic variation of the V3 region sequences of 28

HIV-infected patients from Paris, France. RESULTS: Comparison of the Parisian V3 loop sequences with other published data indicates that the range of diversity in France is included within that of a large group that contains sequences from North America, the rest of Europe, Japan, India and Africa. Variability appears to be lower in the V3 loop than in its flanking regions. Five out of the six putative **N-linked glycosylation** sites show preferential alterations to charged amino acids. We report two motifs at the tip of the loop that have not been described previously. CONCLUSIONS: The structural homogeneity and the wide geographic representation of the major V3 group suggests that a common strategy could be applied to a large proportion of isolates in the development of a broad-spectrum **HIV** vaccine.

L23 ANSWER 46 OF 49 MEDLINE on STN
93248254. PubMed ID: 8483933. Proposed atomic structure of a truncated **human immunodeficiency virus** glycoprotein **gp120** derived by molecular modeling: target CD4 recognition and docking mechanism. Gabriel J L; Mitchell W M. (Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA 19140.) Proceedings of the National Academy of Sciences of the United States of America, (1993 May 1) Vol. 90, No. 9, pp. 4186-90. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The atomic structure of a truncated glycoprotein **gp120** from **human immunodeficiency virus** 1 (**HIV**-1) that contains the principal **neutralizing** antigenic sites and the CD4 binding domain has been derived by molecular dynamics and calculation of potential energy using the DREIDING force field. The resultant N-**glycosylated** molecular model is consistent with known properties of **gp120** and docks with CD4 with a substantial reduction in the sum of the internal potential energies of the individual proteins (delta E = -200 kcal/mol). The primary mechanism of recognition and binding is the insertion of the solvent-accessible Phe-43 of CD4 into a **gp120** solvent-accessible acceptor pit formed by Trp-427, Tyr-435, and the high-mannose oligosaccharide **N-linked** to Asn-230. delta E for the nonglycosylated complex is reduced significantly (-75 kcal/mol). Binding is by pi-pi* interactions of the aromatic groups forming a hydrophobic, thermodynamically stable **environment** for these functional noncovalent bonding participants. This model for **gp120** provides a theoretical basis for the evaluation of **HIV** molecular pathogenesis involving the **env** proteins, the analysis of conformation on functional immune response of the host, and the design of nonproteinaceous inhibitors specific for the CD4 binding site on **gp120**.

L23 ANSWER 47 OF 49 MEDLINE on STN
91162753. PubMed ID: 1705994. Strain-specific **neutralizing** determinant in the transmembrane protein of **simian immunodeficiency virus**. Kodama T; Burns D P; Silva D P; Veronese F D; Desrosiers R C. (New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772.) Journal of virology, (1991 Apr) Vol. 65, No. 4, pp. 2010-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Monoclonal antibody SF8/5E11, which recognizes the transmembrane protein (TMP) of **simian immunodeficiency virus** of macaque monkeys (SIVmac), displayed strict strain specificity. It reacted with cloned and uncloned SIVmac251 but not with cloned SIVmac142 and SIVmac239 on immunoblots. This monoclonal antibody **neutralized** infection by cloned, cell-free SIVmac251 and inhibited formation of syncytia by cloned SIVmac251-infected cells; these activities were specific to cloned SIVmac251 and did not occur with the other viruses. Site-specific mutagenesis was used to show that TMP amino acids 106 to 110 (Asp-Trp-Asn-Asn-Asp) determined the strain specificity of the monoclonal antibody. This strain-specific **neutralizing** determinant is located within a variable region of SIVmac and **human immunodeficiency virus** type 2 (**HIV**-2) which includes

conserved, clustered sites for **N-linked glycosylation**. The determinant corresponds exactly to a variable, weak **neutralizing** epitope in **HIV**-1 TMP which also includes conserved, clustered sites for **N-linked glycosylation**. Thus, the location of at least one **neutralizing** epitope appears to be common to both SIVmac and **HIV**-1. Our results suggest a role for this determinant in the viral entry process. Genetic variation was observed in this **neutralizing** determinant following infection of a rhesus monkey with molecularly cloned SIVmac239; variant forms of the strain-specific, **neutralizing** determinant accumulated during persistent infection in vivo. Selective pressure from the host immune response in vivo may result in sequence variation in this **neutralizing** determinant.

L23 ANSWER 48 OF 49 MEDLINE on STN
91056552. PubMed ID: 2243378. Analysis of sequence diversity in hypervariable regions of the external glycoprotein of **human immunodeficiency virus** type 1. Simmonds P; Balfe P; Ludlam C A; Bishop J O; Brown A J. (Department of Genetics, University of Edinburgh, Scotland.) Journal of virology, (1990 Dec) Vol. 64, No. 12, pp. 5840-50. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Nucleotide sequences in three hypervariable regions of the **human immunodeficiency virus** type 1 (**HIV**-1) **env** gene were obtained by sequencing provirus present in peripheral blood mononuclear cells of **HIV**-infected individuals. Single molecules of target sequences were isolated by limiting dilution and amplified in two stages by the polymerase chain reaction, using nested primers. The product was directly sequenced to avoid errors introduced by Taq polymerase during the amplification process. There was extensive variation between sequences from the same individual as well as between sequences from different individuals. Interpatient variability was markedly less in individuals infected from a common source. A high proportion of amino acid substitutions in the hypervariable regions altered the number and positions of potential **N-linked glycosylation** sites. Sequences in two hypervariable regions frequently contained short (3- to 15-bp) duplications or deletions, and by amplifying peripheral blood mononuclear cell DNA containing 10(2) or 10(3) proviral molecules and analyzing the product by high-resolution electrophoresis, the total number and abundance of distinct length variants within an individual could be estimated, providing a more comprehensive analysis of the variants present than would be obtained by sequencing alone. Sequences from many individuals showed frequent amino acid substitutions at certain key positions for **neutralizing**-antibody and cytotoxic T-cell recognition in the immunodominant loop. The rates of synonymous and nonsynonymous nucleotide substitution in the region of this and flanking regions indicate that strong positive selection for amino acid change is operating in the generation of antigenic diversity.

L23 ANSWER 49 OF 49 MEDLINE on STN
90244398. PubMed ID: 1692349. Inhibition of **human immunodeficiency virus** (**HIV**) infection in vitro by anticarbohydrate monoclonal antibodies: peripheral **glycosylation** of **HIV envelope** glycoprotein **gp120** may be a target for virus **neutralization**. Hansen J E; Clausen H; Nielsen C; Teglbjaerg L S; Hansen L L; Nielsen C M; Dabelsteen E; Mathiesen L; Hakomori S I; Nielsen J O. (Department of Infectious Diseases, Hvidovre Hospital, Denmark.) Journal of virology, (1990 Jun) Vol. 64, No. 6, pp. 2833-40. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Carbohydrate structures are often involved in the initial adhesion of pathogens to target cells. In the present study, a panel of anticarbohydrate monoclonal antibodies (MAbs) was tested for their ability to inhibit in vitro **human immunodeficiency virus** infectivity. MAbs

against three different N- and **O-linked** carbohydrate epitopes (LeY, A1, and sialyl-Tn) were able to block infection by cell-free virus as well as inhibit syncytium formation. Inhibition of virus infectivity was independent of virus strain (HTLVIIIB or patient isolate SSI-002), the cell line used for virus propagation (H9 or MT4), and the cell type used as the infection target (MT4, PMC, or selected T4 lymphocytes). Inhibition was observed when viruses were preincubated with MAbs but not when cells were preincubated with MAbs before inoculation, and the MAbs were shown to precipitate 125I-labeled **gp120**. The MAbs therefore define carbohydrate structures expressed by the viral **envelope** glycoprotein **gp120**, indicating that glycans of the viral **envelope** are possible targets for immunotherapy or vaccine development or both.

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=> d his

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L1           14 S E4

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L2           12 S E3

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L3           58 S E3-E5
L4           50 S L3 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS OR SIV OR SIMIAN
L5           35 S L4 AND (ENV? OR GP160 OR GP120 OR GP41)

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L8        12700 S L7 AND GLYCOSYLAT?
L9         4633 S L8 AND (N-LINKED OR O-LINKED)
L10        3810 S L9 AND (NEUTRALIZ?)
L11         174 S L10 AND (ENV?/CLM OR GP160/CLM OR GP120/CLM OR GP41/CLM)
L12          14 S L11 AND (N-LINKED/CLM OR O-LINKED/CLM)
L13          12 S L12 NOT L1

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L15        2163 S L14 AND (ENV? OR GP160 OR GP120 OR GP41)
L16          61 S L15 AND GLYCOSYLAT?
L17           6 S L16 AND (N-LINKED OR O-LINKED)
L18           4 S L17 NOT L2

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L20       15871 S L19 AND (ENV? OR GP160 OR GP120 OR GP41)
L21         503 S L20 AND GLYCOSYLAT?
L22         157 S L21 AND (N-LINKED OR O-LINKED)
L23          49 S L22 AND (NEUTRALIZ?)

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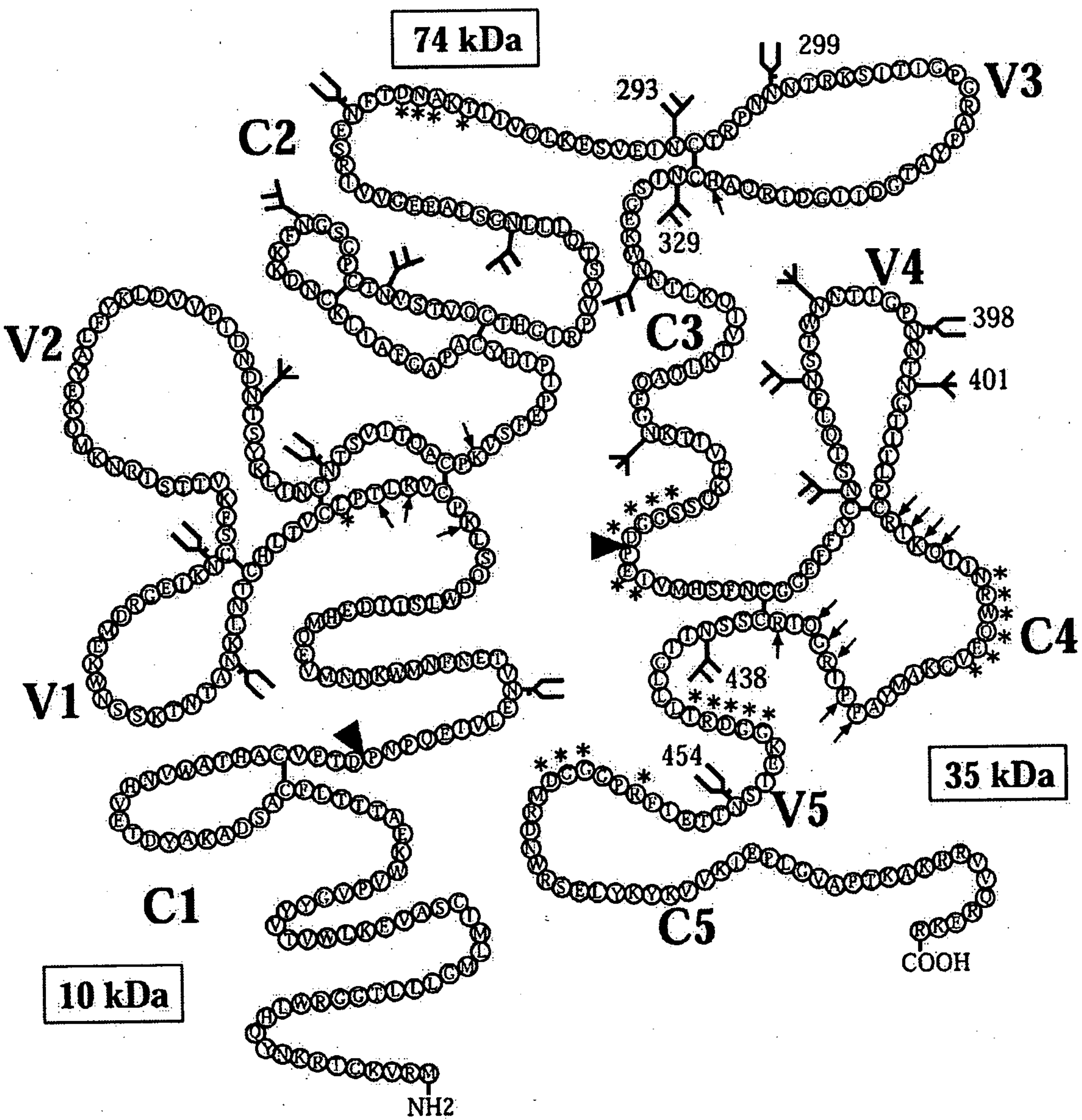
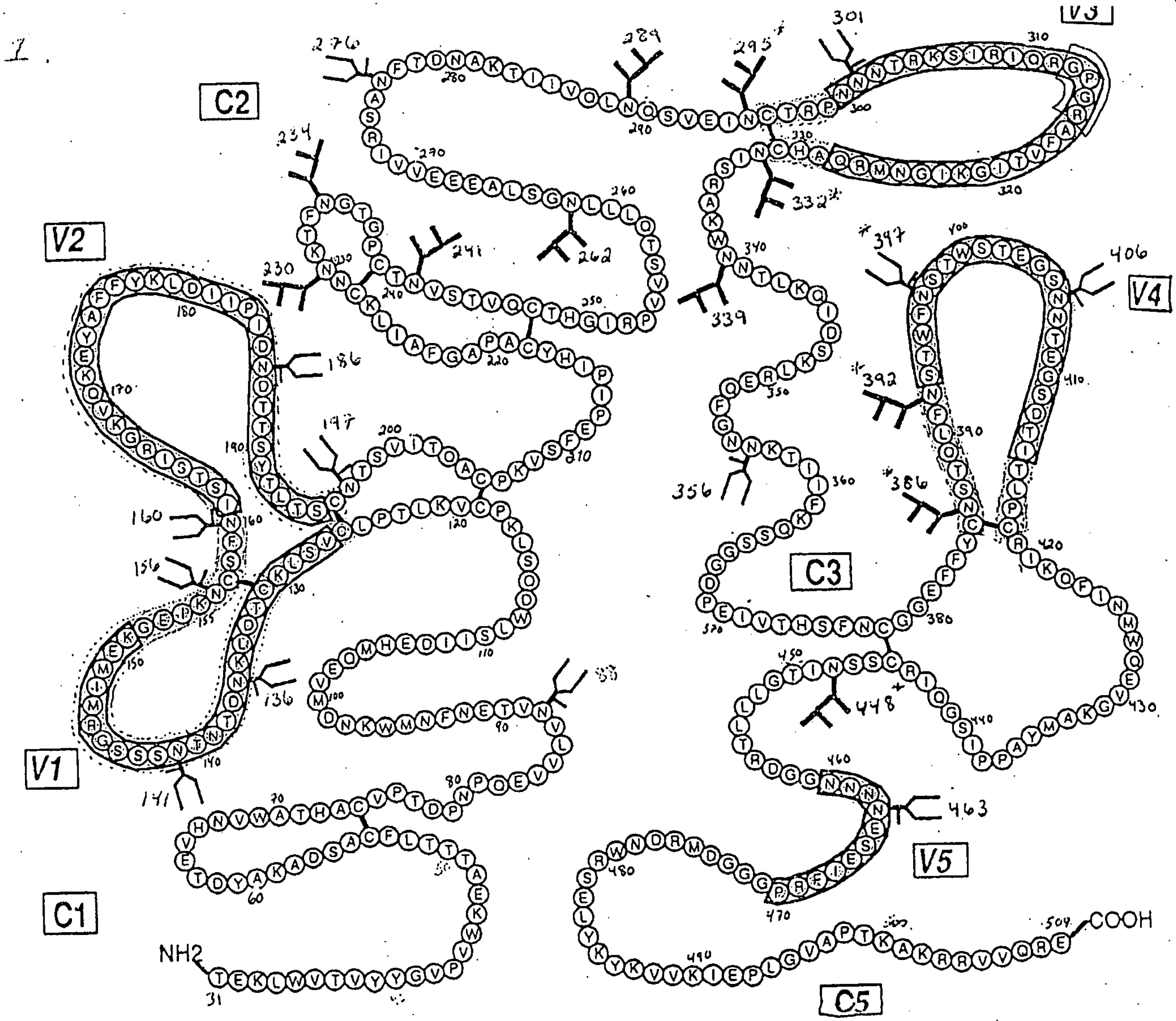


FIG. 1. Schematic representation of SF162 gp120 showing the conserved regions (C1-C5) and the variable loops (V1-V5), based on the sequence of SF162 from Cheng-Mayer et al. (16) and figure adapted from Leonard et al. (49). The positions and types of potential N-linked glycosylation sites are indicated as follows: Y, high-mannose or hybrid glycan; Ψ, complex glycan; and Υ, unknown type of glycan. Arrowheads indicate the positions where glacial acetic acid cleaves gp120 to produce three fragments of approximately 10, 35, and 74 kDa; arrows indicate amino acids outside the V3 loop that are involved in coreceptor binding (81, 82); asterisks indicate amino acids involved in CD4 binding (43).

.0α

Figure 1.

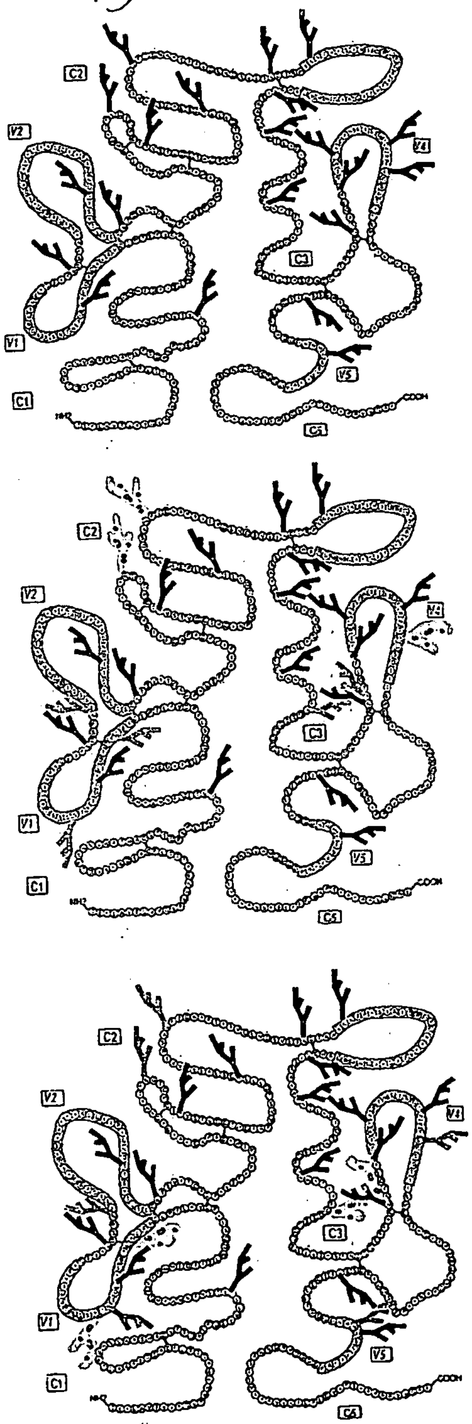


2G12 epitope

' high mannose

. complex

Figure 3



Early (21)

- 21 original sites

Mid (23)

- 5 sites are added
- ~~3 are removed~~

Late (23)

- 4 sites are added
- Of the 5 sites added on the mid virus, ~~4 are now removed~~

Figure 4

A141 alignment of V1/V2

```
SF162        CVTLHCTNLK NATNTKSSN- WKEMDRGEIK NCSFKVTTSI RNKMQKEYAL FYKLDVVPID NDNTS--YKL INC
A141-35-1    .......... .......... .......... .......... .......... .......... .......... ...
A141-35-2    .......... .T........ .......... .......... .......... .......... .......... .S.
A141-35-3    .......... .......... .......... .......... .......... .......... .......... ...
A141-35-4    .......... .T........ .......... .......... .....R.... .......... .......... ...
A141-35-10   .......... .T........ .......... .......... .......... .......... .......... .S.
A141-35-11   .......... .......... .......... .......... .......... .......... .......... ...
A141-35-12   .......... .......... .......... .......... .......... .......... .......... ...
A141-35-13   .......... .......... .......... .......... .......... .......... .......... ...
A141-35-14   .......... .......... .......... .......... .......... .......... .......... ...

SF162        CVTLHCTNLK NATNTKSSN- WKEMDRGEIK NCSFKVTTSI RNKMQKEYAL FYKLDVVPID NDNTS--YKL INC
A141-56-1    .......... .T........ .......... .......... .......... .......... .......... ...
A141-56-2    .......... ........D. .......... ....N..... .......... .......... .......... ...
A141-56-3    .......... .T......D. .......... .......... .......... .......... .......... ...
A141-56-4    .......... .T........ .......... .......... .......... .......... .......... ...
A141-56-5    .......... .T........ .......... .......... .......... .......... .......... ...
A141-56-10   .......... .T........ .......... .......... .......... .......... .......... ...
A141-56-11   .......... ........D. .......... ....N..... .......... .......... .......... ...
A141-56-12   .......... ........D. .......... ....N..... .......... .......... .......... ...
A141-56-13   .......... .T........ .......... .......... .......... .......... .......... ...
A141-56-14   .......... .T........ .......... .......... .......... .......... .......... ...

SF162        CVTLHCTNLK NATNTKSSN- WKEMDRGEIK NCSFKVTTSI RNKMQKEYAL FYKLDVVPID NDNTS--YKL INC
A141-84-1    ...N..D.R. .....N..S. EGKVE..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-84-2    ...N..D.R. .....N..S. EGKVE..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-84-3    ...N..D.R. .....N..S. EGKVE..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-84-4    ...N...... .T........ .......... .......... .......... .......... .......... ...
A141-84-5    ...N..D.R. .....N..S. EGKVE..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-84-6    .......... .....N.... .......... ....N..... .......... .......... ....--TS.. ...
A141-84-7    ...N...... .T........ .......... .......... .......... .......... .......... ...
A141-84-8    ...N..D.R. .....N..S. EGKVE..... ....NI.... .D.V...... .......... .....PS.RW .S.
A141-84-9    ...N..D.R. .....N..S. EGKVE..... ....NI.... .D.V...... .......... .....TS.R. .S.

SF162        CVTLHCTNLK NATNTKSSN- WKEMDRGEIK NCSFKVTTSI RNKMQKEYAL FYKLDVVPID NDNTS--YKL INC
A141-117-1   ...N..D.R. .....N..S. EGK.E..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-117-2   ...N..D.R. .....N..S. EGK.E..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-117-3   ...N..D.R. .....N..S. EGK.E..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-117-4   ...N..D.R. .....N..S. EGK.E..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-117-5   ...N..D.R. .....N..S. EGKVE..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-117-6   .......... .......... .......... .......... .......... .......... .......... ...
A141-117-7   ...N..D.R. .....N..S. EGK.E..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-117-8   ...N..D.R. .....N..S. EGK.E..... ....NI.... .D.V...... .......... .....TS.R. .S.
A141-117-9   .......... ...[.].T... .....G.... ....N..... .......... .......... .......... ...
A141-117-10  ...N...... .T...E.... .......... .......... .......... .......... .......... ...
A141-117-11  ..S.K..D.. .D...N..SG RMI.EK.... ....NIS... .D.V.....F .....I.... .--.....R. .S.
A141-117-12  ...N..D.R. .....N..S. EGK.E..... ....NI.... .D.V...... .......... ...TS.R... .S.
A141-117-13  ..S.K..D.. .D...N..SG RMI.EK.... ....NIS... .D.V.....F .....I.... .--.....R. .S.
A141-117-14  .......... ........D. .......... ....N..... .......... .......... .......... ...

SF162        CVTLHCTNLK NATNTKSSN- WKEMDRGEIK NCSFKVTTSI RNKMQKEYAL FYKLDVVPID NDNTS--YKL INC
A141-215-1   .......... ........D. .......... ....N..... .......... .......... .......... ...
A141-215-2   .......... ...[.].T... .....G.... ....N..... .......... .......... ........N. ...
A141-215-3   .......... ........D. .......... ....N..... .......... .......... .......... ...
A141-215-5   .......... ........D. .......... ....N..... .......... .......... .......... ...
A141-215-12  .......... ........D. .......... ....N..... .......... .......... .......... ...
A141-215-13  .......... ...[.].T... .....G.... ....N..... .......... .......... ........N. ...
A141-215-14  .......... ...[.].T... .....G.... ....N..... .......... .......... ........N. ...
A141-215-15  .......... ........D. .......... ....N..... .......... .......... .......... ...
```

Figure 5

A141 alignment of V4

```
SF162        CNSTQLFN-- ----STWNN- ---TIGPNNT NG--TITLPC
A141-35-1    .......... .......... .......... ..........
A141-35-2    .......... .......... .......... .R........
A141-35-3    .......... .......... .......... ..........
A141-35-4    .......... .......... .......... ..........
A141-35-10   .......... .......... .......... ..........
A141-35-11   .......... .......... .......... ..........
A141-35-12   .......... .......... .......... ..........
A141-35-13   .......... .......... .......... ..........
A141-35-14   .......... .......... .......... ..........

SF162        CNSTQLFN-- ----STWNN- ---TIGPNNT NG--TITLPC
A141-56-1    .......... .......... .......... ..........
A141-56-2    .......... .......... .......... ..........
A141-56-3    .......... .......... .......... ..........
A141-56-4    .......... .......... .......... ..........
A141-56-5    .......... .......... .......... ..........
A141-56-10   .......... .......... .......... ..........
A141-56-11   .......... .......... .......... ..........
A141-56-12   .......... .......... .......... ..........
A141-56-13   .......... .......... .......... ..........
A141-56-14   .......... .......... .......... ..........

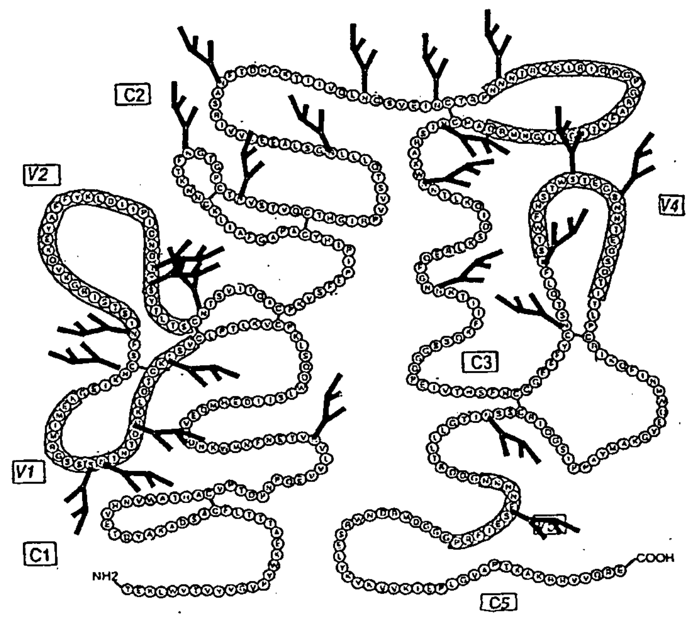
SF162        CNSTQLFN-- ----STWNN- ---TIGPNNT NG--TITLPC
A141-84-1    ........ST WMFN....D. ....KES.D. E...N.....
A141-84-2    ........ST WMFN....D. ....KES.D. E...N.....
A141-84-3    ........ST WMFN....D. ....KES.D. E...N.....
A141-84-4    .......... .......... .......... ..........
A141-84-5    ........ST WMFN....D. ....KES.D. E...N.....
A141-84-6    ........ST WMFN....D. ....KES.D. E...N.....
A141-84-7    .......... .......... .......... ..........
A141-84-8    .......... .......... .......... ..........
A141-84-9    ........ST WMFN....D. ....KES.D. E...N.....

SF162        CNSTQLFN-- ----STWNN- ---TIGPNNT NG--TITLPC
A141-117-1   ........ST WMFN....D. ....KES.D. E...N.....
A141-117-2   ........ST WMFN....D. ....KES.D. E...N.....
A141-117-3   ........ST WMFN....D. ....KES.D. E...N.....
A141-117-4   ........ST WMFN....D. ....KES.D. E...N.....
A141-117-5   ........ST WMFN....D. ....KES.D. E...N.....
A141-117-6   .......... .......... .......... ..........
A141-117-7   ........ST WMFN....D. ....KES.D. E...N.....
A141-117-8   ........ST WMFN....D. ....KES.D. E...N.....
A141-117-9   .......... .......S.. .......... ..........
A141-117-10  .......... .......... .......... ..........
A141-117-11  .......... .......F.S TWS.E.S... E.SD......
A141-117-12  ........ST WMFN....D. ....KES.D. E...N.....
A141-117-13  .......... .......F.S TWS.E.S... E.SD......
A141-117-14  .......... .......... .......... ..........

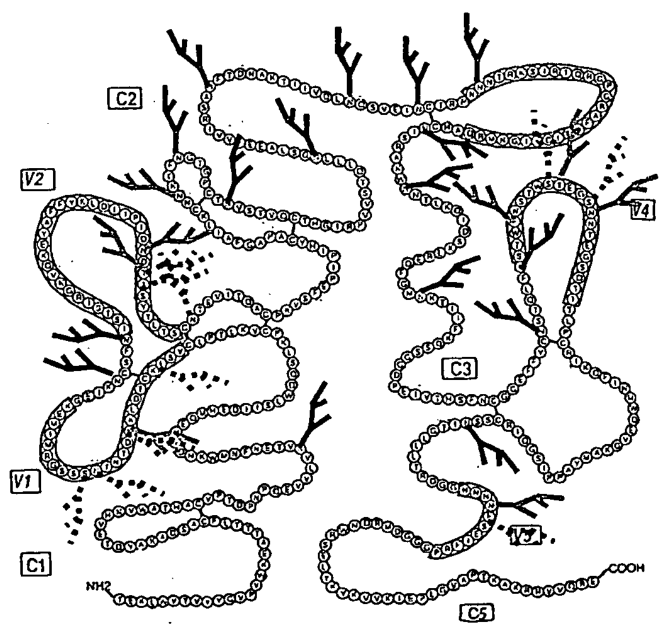
SF162        CNSTQLFN-- ----STWNN- ---TIGPNNT NG--TITLPC
A141-215-1   .......... .......... .......... ..........
A141-215-2   .......... .......... .......... .E........
A141-215-3   .......... .......... .......... ..........
A141-215-5   .......... .......... .......... ..........
A141-215-12  .......... .......... .......... ..........
A141-215-13  .......... .......... .......... ..........
A141-215-14  .......... .......... .......... ..........
A141-215-15  .......... .......... .......... ..........
```

Figure 2

Clade B
NSI



Clade B
SI



hypothesized that the NtAbs work via recognition of conserved *structures* that are shared among the different subtypes. Indeed, sera from HIV-infected patients were shown to contain conformation-dependent NtAbs that can recognize sequentially diverse HIV subtypes (Steimer *et al.* 1991). The Env has been shown to change conformation in the process of binding to the receptor CD4, exposing neutralization determinants (Sullivan *et al.* 1998).

In SIV-infected macaques, the extent of viral Env evolution and the titer of NtAb correlate with the extent of persistent viremia (Hirsch *et al.* 1998). In HIV infection, the development of novel NtAbs during antiviral drug suppression requires the production of at least measurable levels of viral antigen (Kimura *et al.* 2002). HIV-infected patients vary in the levels of their intra- and intersubtype-specific NtAbs, and the extent of Env variation is also variable. As the virus evolves during the course of an HIV infection, the Env sequence changes and frequently alters both alters the number and the location of N-linked glycosylation sites. One of the key mutations in the HIV Env protein that is associated with neutralization escape is the addition and deletion of carbohydrate residues and/or charged residues in or near the V1 and V2 hypervariable regions and in the upstream C3 region (Wang *et al.* 2002). It has been shown that glycosylation mutants of SIV arising later in infection and that add carbohydrate can be more resistant to neutralization (Chackerian *et al.* 1997) and more pathogenic *in vivo* (Kimata *et al.* 1999). Hypervariable regions are involved in binding to coreceptors, and changes to these regions have been shown to influence the conformation of Env affecting subunit association, syncytium formation, and recognition by a neutralizing antibody (Sullivan *et al.* 1993; Wyatt *et al.* 1995).

Prior HIV vaccines have employed Env immunogens with limited clinical success. Soluble Envelopes (gp120) act as excellent immunogens to generate anti-Env antibodies, but typically generate only low levels of homologous NtAbs, sometimes intrasubtype NtAbs, but only very rarely intersubtype NtAbs (Haigwood *et al.* 1992; Mascola *et al.* 1996). To drive the immune response toward conserved regions, it was logical to

immunize with an Env devoid of variable sequences, and such experiments were attempted with recombinant Env subunit proteins. It was demonstrated that at least one variable region was necessary to elicit detectable NtAbs in experimental animals (Haigwood *et al.* 1990). Failure to raise NtAbs to conserved regions was probably due to the denature immunogen, which did not preserve the shape of the remaining conserved, nonvariable regions. Approaches to overcoming the poor immunogenicity of conserved conformational determinants have included the use of primary virus Env gp120, and soluble oligomeric Envs (Stamatos et al. 1998), stabilized by disulfide bonds (Binley *et al.* 2000) or shortened to make Env gp140 by removing the transmembrane domain of the TM protein gp41 (Stamatatos *et al.* 2000). None of these approaches has resulted in immunogens that elicit broad immunity at the level seen in HIV-infected patients with high-level intersubtype NtAbs. Another approach to altering the repertoire of NtAbs was to draw immunity away from the immunogenic variable region V3 by masking the region with carbohydrate (Garrity *et al.* 1997). The masking succeeded in driving immunity to other variable regions that were more immunogenic than in the wild type Env, but no cross-neutralizing responses were obtained.

The Envs from late stage patients who have antibodies that can neutralize intra- and intersubtype variants may act as poor immunogens, presumably because the additional carbohydrate (CHO) occludes or limits exposure to the NtAb determinants on the envelope. Removal of key CHO residues results in immunogens that are more effective in eliciting intrasubtype NtAbs. This as been accomplished by removal of multiple N-linked carbohydrate sites in the SIV Env (Mori *et al.* 2001) and by removal of V2-associated carbohydrate in the HIV Env by deletion (Stamatatos and Cheng-Mayer 1998). These changes in Envelope make it more sensitive to neutralization in vitro (Stamatatos and Cheng-Mayer 1998; Stamatos *et al.* 1998); however, these immunogens are still only partially effective at generating intersubytpe NtAbs (Barnett *et al.* 2001). An alternative approach to broadening responses has been to immunized with Envelopes derived from different subtypes, resulting in some NtAbs in mice, but no evidence of

significant cross-subtype immunity (Ljungberg *et al.* 2002). Recent efforts to expose more conserved determinants by fixing the conformation of Envelope have met with some success. Cross-linked Envelope and CD4 complexes have been shown to elicit broad intersubtype NtAbs in rhesus macaques (Fouts *et al.* 2002). Immunization of humans with their CD4 molecule may raise unwanted immune responses, due to the potential for autoimmune recognition of CD4-bearing cells. Therefore alternatives to presenting conserved conformational regions of Envelope are desirable.

Summary of the Invention

This invention provides a sequential vaccination protocol that directs the immune response away from the highly immunogenic variable regions toward the conserved determinants, thereby promoting generation of intra-subtype and inter-subtype primary HIV neutralizing antibodies (NtAbs). This result mimics the pattern of variants that arise *in vivo* but shortens the time needed to achieve the clinically beneficial result.

The subject vaccination protocol features immunization with a first HIV envelope immunogen having a minimum number of N- and O-linked glycosylation sites, followed by one or more booster immunizations with second HIV envelope immunogens having more glycosylation sites than the first immunogen.

The first immunogen preferably has from about 24 to 26 glycosylation sites in gp160, or 20 in gp120 and 4 in gp41. The following glycosylation sites are presently considered desirable: in gp120, positions 88, 130, 136, 156, 160, 197, 241, 262, 276, 296, 301, 332, 339, 355 or 356, 386, 392, 396 or 397, 405 or 406, 448, 463; and in gp41, positions 611, 616, 625, 637. This first immunogen may be derived from a natural clone from a primary HIV infection, or an engineered Envelope. In this manner the first immunogen (or second) can include gp160, gp140, or gp120, or likewise engineered constructs.

The second immunogen has at least one and preferably three or more additional glycosylation sites than the first immunogen. These additional sites may be located within hypervariable regions V1, V2, V3, V4, or V5 or constant region C2 or possibly

regions 1 and 2 (V1/V2). Sequences are compared with the published HIV-SF162 *env* sequence. Sequence alignments are standard and show a "." where there is identity and a new letter for any changes. Boxed "N" residues indicate those that are part of the canonical N-X-(S,T) N-linked glycosylation site. Sequences are for day 35 (8 clones); day 56 (10 clones); day 84 (9 clones); day 117 (14 clones); day 215 (8 clones).

FIGURE 5 shows the translated amino acid sequence of HIV Envelope from macaque A141 as in Figure 4. This alignment is of the 4^{th} Variable region (V4).

Detailed Description of the Preferred Embodiment

Our laboratory set out to determine whether changes in carbohydrate are important for altering the immunogenicity of Env. Relatively little data comparing early and late HIV Env sequences and changes over time from the same patients are available. We have analyzed the primary non syncytium-inducing (NSI) and late stage syncytium-inducing (SI) full length Env sequences from HIV-1 infected patients from the Los Alamos database (http://hiv-web.lanl.gov/content/hiv-db/ALIGN_CURRENT/ALIGN-INDEX.html). These sequences are not from the same patients, but represent average changes in sequences that are common to most NSI and SI viral Envelopes sequenced to date. Envelopes from primary HIV infections (NSI sequences) typically have less than about 24 N-linked glycosylation sites. The envelopes of late stage patients or SI virus sequences typically contain about 25 or more glycosylation sites. We note that changes result from both loss and gain of glycosylation sites, resulting in repositioning of carbohydrate in key regions. The glycosylation sites found in NSI and SI isolates are summarized in the table below (Table 1). Sequences were numbered according to the HXBc2 sequence, and occurrence of N-X-(S,T) at specific sites was scored for each sequence. Numbers listed indicate scores near 1. A summary of the primary data is provided in graphic form in Figure 2. In this case, no information is available about neutralization sensitivity or the presence of neutralizing antibodies in the sera of the infected patients.

chain reaction (PCR) and Taq polymerase. Primers that amplify Envelope are situated outside the Envelope coding region and prime the entire length of the gene. Primers that amplify Envelope from many different HIV sequences are designed to bind to conserved regions of the viral genome outside the envelope coding region. In many cases, it may be necessary to use two rounds of PCR to obtain the Env sequence. In this case, the second set of primers will be situated inside the outer primers to make a "nested set" of sequences, where the first set will amplify a slightly larger product and the second nested set a smaller product.

Selection or Engineering of the 1^{ST} AND 2^{ND} Immunogens and Testing Criteria

By analysis of published and cloned sequences, the minimum number of N-linked glycosylation sites that are present on early infecting isolates has been deduced. These are usually but not exclusively located at positions note below (numbering system follows the convention of numbering from the HXB2 cloned sequence). The exact locations of these sites may vary slightly from one HIV isolate to another:

SU gp120: 88, 130, 136, 156, 160, 197, 241, 262, 276, 296, 301, 332, 339, 355 or 356, 386, 392, 396 or 397, 405 or 406, 448, 463; n=20

TM gp41: 611, 616, 625, 637; n=4

If a cloned envelope gene encodes approximately this number of sites, it may be used as an immunogen directly. If the gene encodes more N-linked sites than approximately 24, then the first immunogen can be derived from the cloned gene by alteration to create appropriate mutations. Additional sites are removed by, for example, site-directed mutagenesis to change Asn residues to Gln residues. The resulting sequence should retain N-X-(S,T) sites at the key positions listed above, or at sites adjacent to these sites.

Second immunogen(s) can then be engineered by the addition of at least one and preferably 3 or more additional N-linked site at positions recommended below, if they are not already present in the first immunogen sequence:

C1: 130, to provide a preferred total of 2 sites

V1: 141, to provide a preferred total of 3 sites

V2: 160 or 186-188, to provide a preferred total of 2 sites

C2: to provide a preferred total of up to 8 sites at positions noted

V3: 1 site preferred at position 301

C3: 362, to provide a preferred total of 4 sites or up to 5 sites

V4: 393, 402, or 409, to provide a preferred total of 5 sites at positions noted

C4: 1 site preferred at position 448

V5: 2 sites preferred at 461 and 463

Gp41: to provide a preferred total of up to 5 sites at positions noted

In some cases it may be necessary to provide more than two different immunogens. Preferably the subsequent immunogens will each bear increasing numbers of glycosylation sites selected from these regions listed above, so that the third variant will bear 26-27 sites and the fourth immunogen will bear 28 sites or more. In general, the first immunogen should bear the least number of N- or O-linked glycosylation sites, and the last immunogen should bear the most. In some cases it may be beneficial to maintain the number of sites in V1, V2, and V4 and to shift the positions of these sites.

USEFUL VACCINATION PROTOCOLS

Useful vaccination protocols may include recombinant vaccines that express the Env protein. Examples of this include recombinant viruses such as poxviruses (vaccinia virus, modified Vaccinia Ankara, and canarypox vectors); recombinant adenoviruses that are replication competent or replication incompetent; recombinant adenovirus-associated virus; Venezuelan Equine Encephalitis viruses; or Vesicualr Stomatatis Viruses, for example. Additional vaccines may include DNA expression vectors typically based on the human cytomegalovirus Immediate Early-I promoter and competent to direct Env expression in mammalian cells ("DNA vaccines") that may be delivered by a variety of routes (intramuscular, intradermal, transdermal, oral, intravaginal, intrarectal, intranasal, etc.). Further, vaccines may consist of recombinant proteins that are delivered in adjuvants or in microspherical biodegradable particles to various sites using intramuscular

regions 1 and 2 (V1/V2). Sequences are compared with the published HIV-SF162 *env* sequence. Sequence alignments are standard and show a "." where there is identity and a new letter for any changes. Boxed "N" residues indicate those that are part of the canonical N-X-(S,T) N-linked glycosylation site. Sequences are for day 35 (8 clones); day 56 (10 clones); day 84 (9 clones); day 117 (14 clones); day 215 (8 clones).

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HIV	**Region of glycosylation site**								
Type	C1	V1	V2	C2	V3	C3	V4	C4	V5	41
NSI	88	136	160	197	301	332	386	448	**(461)**	611
24	130	141	**(188)**	234		339	392		463-4	616
sites		156		241		355/6	396/7			625
				262		(362)	405/6			637
				276						
				289						
				295						
SI	88	136	160	197	301	332	386	448	463	611
25	130	141	**(186)**	**230**		339	392			616
sites		156		234		356	(396/7)			625
				241		(362)	**(402)**			637
				262			(406)			**816**
				276						
				289						
				295						

Table 1. Amino acid residues in constant (C) and variable (V) regions of Envlope that are potential N-linked or O-linked glycosylation sites. Comparison of 25 NSI and 16 SI HIV B subtype isolates from the Los Alamos Database. Positions noted are those that are present in all or nearly all sequences compared. Numbers in parentheses are present less than 100% time; underlined numbers are those that differ between NSI and SI.

The chimeric virus SHIV bears the HIV *env* gene in the backbone of the SIV genome. SHIV infection of macaques has produced a number of SHIV isolates that cause pathogenesis in the macaque in a time frame of 1 year or less. These macaques are

subject to sequence diversification and develop NtAbs against viral variants, just as HIV-infected humans do. Comparison of the data from SHIV-infected macaques and HIV-infected humans shows remarkable conservation of glycosylation sites, and it also allows a comparison of sites that are altered over time in both types of infection. We have used the polymerase chain reaction (PCR) and HIV-1 *env*-specific primers to amplify the envelope gene gp160 or gp120 from DNA obtained from peripheral blood mononuclear cells of infected macaques. From these sequences, we inferred the amino acid sequence and analyzed these sequences for changes, and particularly for changes in N-linked glycosylation sites. The glycosylation site data summarized in Table 2 are from the first year samples from a SHIV-infected macaque with moderate disease progression and evidence of significant increases in magnitude and breadth of the NtAb response between day 56 and day 84. Changes to the glycosylation pattern are underlined in the day 84-117 data, showing the acquisition of 5 sites during quasispecies differentiation. Additional data are shown in Figures 3-5. We propose that the changes observed in glycosylation pattern sites are contributing to the broadening of the neutralizing antibody response in this animal.

SHIV	Region of glycosylation site									
Days	C1	V1	V2	C2	V3	C3	V4	C4	V5	41
35-56	88	136		197	301	332	386	448	463	611
			188	234		339	392			616
		156		241		355				625
				262			396			637
				276			406			
				295			409			
84-117	88	136	**<u>160</u>**	197	301	332	386	448	463	611
	<u>130</u>	**<u>141</u>**	188	234		339	392			616
		156		241		355	**<u>393</u>**			625
				262		**<u>362</u>**	396			637
				276			406			
				289			409			
				295						

Table 2. Amino acid residues in constant (C) and variable (V) regions of SHIV Envlope that are potential N-linked or O-linked glycosylation sites. Summary of comparison of multiple Envelope sequences from macaque A141, infected with SHIV-SF162P4, at days 35 and 56 (early); at days 84 and 117 (middle). Positions noted are those that are present in all or nearly all sequences compared. Numbers in parentheses are present less than 100% time; underlined numbers are those are present at the later time point and not present at the earlier time point.

PRODUCTION of ENV IMMUNOGENS (GENES AND PROTEINS)

Envelope genes can be obtained from patient virus samples by pelleting virus from plasma and extracting RNA, reverse transcribing the RNA into DNA, and specifically amplifying the Envelope DNA using DNA primers and the polymerase

Citing References

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AB Immunization by the SF162gp140 or the DeltaV2gp140 **HIV**-1 **envelope** proteins results in the generation of strong homologous **neutralizing** antibodies (NAbs) that offer similar degree of protection from disease-development to macaques challenged with homologous virus. These two immunogens elicit weak cross-reactive NAbs and their effectiveness against heterologous challenge is currently unknown. To examine this issue, we immunized macaques with SIVGag p55 and either the SF162gp140 or the DeltaV2gp140 and challenged them intravenously with SHIV-89.6P. All animals became infected but previous immunization with SF162gp140 accelerated the development of anti-SHIV89.6P **neutralizing** antibody responses following infection. DeltaV2gp140 is derived from SF162gp140 following the deletion of 30 amino acids and one **N-linked glycosylation** site from the V2 loop. Our results suggest that even small differences in **HIV Envelope** immunogen structure can affect the **neutralizing** antibody responses generated following infection.

2004536680. PubMed ID: 15507649. Evolutionary dynamics of the glycan shield of the **human immunodeficiency virus envelope** during natural infection and implications for exposure of the 2G12 epitope. Dacheux Laurent; Moreau Alain; Ataman-Onal Yasemin; Biron Francois; Verrier Bernard; Barin Francis. (Laboratoire de Virologie, CHU Bretonneau, 37044 Tours cedex, France.) Journal of virology, (2004 Nov) Vol. 78, No. 22, pp. 12625-37. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Elucidation of the kinetics of exposure of **neutralizing** epitopes on the **envelope** of **human immunodeficiency virus** type 1 (**HIV**-1) during the course of infection may provide key information about how **HIV** escapes the immune system or why its **envelope** is such a poor immunogen to induce broadly efficient **neutralizing** antibodies. We analyzed the kinetics of exposure of the epitopes corresponding to the broadly **neutralizing** human monoclonal antibodies immunoglobulin G1b12 (IgG1b12), 2G12, and 2F5 at the quasispecies level during infection. We studied the antigenicity and sequences of 94 full-length **envelope** clones present during primary infection and at least 4 years later in four **HIV**-1 clade B-infected patients. No or only minor exposure differences were observed for the 2F5 and IgG1b12 epitopes between the early and late clones. Conversely, the **envelope** glycoproteins of the **HIV**-1 quasispecies present during primary infection did not expose the 2G12 **neutralizing** epitope, unlike those present after several years in three of the four patients. Sequence analysis revealed major differences at potential **N-linked glycosylation** sites between early and late clones, particularly at positions known to be important for 2G12 binding. Our study, in natural mutants, confirms that the **glycosylation** sites N295, N332, and N392 are essential for 2G12 binding. This study demonstrates the relationship between the evolving "glycan shield " of **HIV** and the kinetics of exposure of the 2G12 epitope during the course of natural infection.

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2004170964. PubMed ID: 15063126. **Neutralization** sensitivity of a simian-**human immunodeficiency virus** (SHIV-HXBc2P 3.2N) isolated from an infected rhesus macaque with neurological disease. Song Byeongwoon; Cayabyab Mark; Phan Ngoc; Wang Liping; Axthelm Michael K; Letvin Norman L; Sodroski Joseph G. (Department of Cancer Immunology and AIDS, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA.)
Virology, (2004 Apr 25) Vol. 322, No. 1, pp. 168-81. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Simian-**human immunodeficiency virus** (SHIV) chimerae, after in vivo passage in monkeys, can induce acquired immunodeficiency syndrome (AIDS)-like illness and death. A monkey infected with the molecularly cloned, pathogenic SHIV-HXBc2P 3.2 exhibited multifocal granulomatous pneumonia as well as progressive neurological impairment characterized by tremors and pelvic limb weakness. SHIV-HXBc2P 3.2N was isolated from brain tissue explants and characterized. Viruses with the **envelope** glycoproteins of SHIV-HXBc2P 3.2N exhibited increased sensitivity to soluble CD4 and several **neutralizing** antibodies compared with viruses with the parental SHIV-HXBc2P 3.2 **envelope** glycoproteins. By contrast, viruses with SHIV-HXBc2P 3.2 and SHIV-HXBc2P 3.2N **envelope** glycoproteins were **neutralized** equivalently by 2G12 and 2F5 antibodies, which are rarely elicited in **HIV**-1-infected humans. A constellation of changes involving both **gp120** and **gp41 envelope** glycoproteins was responsible for the difference in susceptibility to **neutralization** by most antibodies. Surprisingly, the gain of an **N-linked glycosylation** site in the **gp41** ectodomain contributed greatly to **neutralization** sensitivity. Thus, the **environment** of the central nervous system, particularly in the context of immunodeficiency, allows the evolution of immunodeficiency viruses with greater susceptibility to **neutralization** by antibodies.

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2004125675. PubMed ID: 15016849. **N-linked glycosylation** of the V3 loop and the immunologically silent face of **gp120** protects **human immunodeficiency virus** type 1 SF162 from **neutralization** by anti-**gp120** and anti-**gp41** antibodies. McCaffrey Ruth A; Saunders Cheryl; Hensel Mike; Stamatatos Leonidas. (Seattle Biomedical Research Institute. Department of Pathobiology, University of Washington, Seattle, Washington 98109, USA.) Journal of virology, (2004 Apr) Vol. 78, No. 7, pp. 3279-95. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We examined how asparagine-linked glycans within and adjacent to the V3 loop (C2 and C3 regions) and within the immunologically silent face (V4, C4, and V5 regions) of the **human immunodeficiency virus** (**HIV**) SF612 **envelope** affect the viral phenotype. Five of seven potential **glycosylation** sites are utilized when the virus is grown in human peripheral blood mononuclear cells, with the nonutilized sites lying within the V4 loop. Elimination of glycans within and adjacent to the V3 loop renders SF162 more susceptible to **neutralization** by polyclonal **HIV**(+)-positive and simian/**human immunodeficiency virus**-positive sera and by monoclonal antibodies (MAbs) recognizing the V3 loop, the CD4- and CCR5-binding sites, and the extracellular region of **gp41**. Importantly, our studies also indicate that glycans located within the immunologically silent face of **gp120**, specifically the C4 and V5 regions, also conferred on SF162 resistance to **neutralization** by anti-V3 loop, anti-CD4 binding site, and anti-**gp41** MAbs but not by antibodies targeting the coreceptor binding site. We also observed that the amino acid composition of the V4 region contributes to the **neutralization** phenotype of SF162 by anti-V3 loop and anti-CD4 binding site MAbs. Collectively, our data support the proposal that the **glycosylation** and structure of the immunologically silent face of the **HIV envelope** plays an important role in defining the **neutralization** phenotype of **HIV** type 1.

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2003416297. PubMed ID: 12954207. Structure-based, targeted deglycosylation of **HIV**-1 **gp120** and effects on **neutralization** sensitivity and antibody recognition. Koch Markus; Pancera Marie; Kwong Peter D; Kolchinsky Peter; Grundner Christoph; Wang Liping; Hendrickson Wayne A; Sodroski Joseph; Wyatt Richard. (Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115, USA.) Virology, (2003 Sep 1) Vol. 313, No. 2, pp. 387-400. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The **human immunodeficiency virus** (**HIV**-1) exterior **envelope** glycoprotein, **gp120**, mediates receptor binding and is the major target for **neutralizing** antibodies. Primary **HIV**-1 isolates are characteristically more resistant to broadly **neutralizing** antibodies, although the structural basis for this resistance remains obscure. Most broadly **neutralizing** antibodies are directed against functionally conserved **gp120** regions involved in binding to either the primary virus receptor, CD4, or the viral coreceptor molecules that normally function as chemokine receptors. These antibodies are known as CD4 binding site (CD4BS) and CD4-induced (CD4i) antibodies, respectively. Inspection of the **gp120** crystal structure reveals that although the receptor-binding regions lack **glycosylation**, sugar moieties lie proximal to both receptor-binding sites on **gp120** and thus in proximity to both the CD4BS and the CD4i epitopes. In this study, guided by the X-ray crystal structure of **gp120**, we deleted four **N-linked glycosylation** sites that flank the receptor-binding regions. We examined the effects of selected changes on the sensitivity of two prototypic **HIV**-1 primary isolates to **neutralization** by antibodies. Surprisingly, removal of a single **N-linked glycosylation** site at the base of the **gp120** third variable region (V3 loop) increased the sensitivity of the primary viruses to **neutralization** by CD4BS antibodies. **Envelope** glycoprotein oligomers on the cell surface derived from the V3 glycan-deficient virus were better recognized by a CD4BS antibody and a V3 loop antibody than were the wild-type glycoproteins. Absence of all four **glycosylation** sites rendered a primary isolate sensitive to CD4i antibody-mediated **neutralization**. Thus, carbohydrates that flank receptor-binding regions on **gp120** protect primary **HIV**-1 isolates from antibody-mediated **neutralization**.

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2003132652. PubMed ID: 12646921. Antibody **neutralization** and escape by **HIV**-1. Wei Xiping; Decker Julie M; Wang Shuyi; Hui Huxiong; Kappes John C; Wu Xiaoyun; Salazar-Gonzalez Jesus F; Salazar Maria G; Kilby J Michael; Saag Michael S; Komarova Natalia L; Nowak Martin A; Hahn Beatrice H; Kwong Peter D; Shaw George M. (Howard Hughes Medical Institute, University of Alabama at Birmingham, 720 South 20th Street, KAUL 816, Birmingham, Alabama 35294-0024, USA.) Nature, (2003 Mar 20) Vol. 422, No. 6929, pp. 307-12. Journal code: 0410462. ISSN: 0028-0836. Pub. country: England: United Kingdom. Language: English.

AB **Neutralizing** antibodies (Nab) are a principal component of an effective human immune response to many pathogens, yet their role in **HIV**-1 infection is unclear. To gain a better understanding of this role, we examined plasma from patients with acute **HIV** infection. Here we report the detection of autologous Nab as early as 52 days after detection of **HIV**-specific antibodies. The viral inhibitory activity of Nab resulted in complete replacement of **neutralization**-sensitive virus by successive populations of resistant virus. Escape virus contained mutations in the **env** gene that were unexpectedly sparse, did not map generally to known **neutralization** epitopes, and involved primarily changes in **N-linked glycosylation**. This pattern of escape, and the exceptional density of **HIV**-1 **envelope glycosylation** generally, led us to postulate an evolving 'glycan shield' mechanism of **neutralization** escape whereby selected changes in glycan packing prevent Nab binding but not receptor binding. Direct support for this model was obtained by mutational substitution showing that Nab-selected alterations in **glycosylation** conferred escape from both autologous antibody and epitope-specific monoclonal antibodies. The evolving glycan shield thus represents a new mechanism contributing to **HIV**-1 persistence in the face of an evolving antibody repertoire.

2002199775. PubMed ID: 11932385. Role of **N-linked** glycans in a **human immunodeficiency virus envelope** glycoprotein: effects on protein function and the **neutralizing** antibody response. Quinones-Kochs Miriam I; Buonocore Linda; Rose John K. (Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510, USA.) Journal of virology, (2002 May) Vol. 76, No. 9, pp. 4199-211. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The **envelope** (**Env**) glycoprotein of **human immunodeficiency virus** (**HIV**) contains 24 N-**glycosylation** sites covering much of the protein surface. It has been proposed that one role of these carbohydrates is to form a shield that protects the virus from immune recognition. Strong evidence for such a role for **glycosylation** has been reported for **simian immunodeficiency virus** (**SIV**) mutants lacking glycans in the V1 region of **Env** (J. N. Reitter, R. E. Means, and R. C. Desrosiers, Nat. Med. 4:679-684, 1998). Here we used recombinant vesicular stomatitis viruses (VSVs) expressing **HIV Env glycosylation** mutants to determine if removal of carbohydrates in the V1 and V2 domains affected protein function and the generation of **neutralizing** antibodies in mice. Mutations that eliminated one to six of the sites for **N-linked glycosylation** in the V1 and V2 loops were introduced into a gene encoding the **HIV** type 1 primary isolate 89.6 **envelope** glycoprotein with its cytoplasmic domain replaced by that of the VSV G glycoprotein. The membrane fusion activities of the mutant proteins were studied in a syncytium induction assay. The transport and processing of the mutant proteins were studied with recombinant VSVs expressing mutant **Env** G proteins. We found that **HIV Env** V1 and V2 **glycosylation** mutants were no better than wild-type **envelope** at inducing antibodies **neutralizing** wild-type **Env**, although an **Env** mutant lacking glycans appeared somewhat more sensitive to **neutralization** by antibodies raised to mutant or wild-type **Env**. These results indicate significant differences between **SIV** and **HIV** with regard to the roles of glycans in the V1 and V2 domains.